Pharmacokinetics of Bevacizumab after Topical, Subconjunctival, and Intravitreal Administration in Rabbits

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Purpose. To investigate the pharmacokinetics of bevacizumab in rabbits for three different routes of administrations: intravitreal injection, subconjunctival injection, and eye drops.

Methods. Pigmented rabbits received bevacizumab in one eye by topical eye drops (1.25 mg/0.05 mL six times daily for the first 7 days), single subconjunctival injection (1.25 mg/0.05 mL), or single intravitreal injection (1.25 mg/0.05 mL). Bevacizumab concentrations in plasma and ocular tissues in the treated and fellow eyes were determined by sandwich enzyme-linked immunosorbent assay at 1, 2, 4, and 12 weeks after administration.

Results. After intravitreal injection in the treated eye, the mean maximum concentrations (C_{max}) of bevacizumab in the iris/ciliary body and retina/choroid were 109,192.6, and 93,990.0 ng/g, respectively, whereas after subconjunctival injection, the C_{max} was 1418.7 and 295.8 ng/g, respectively. In the fellow eyes, when the drug was administered by intravitreal injection, the C_{max} was 753.6 ng/g in the iris/ciliary body and 224.2 ng/g in the retina/choroid and by subconjunctival injection was 1192.9 and 187.0 ng/g, respectively. With eye drops, only a small level of bevacizumab was detected in the iris/ciliary body and retina/choroid. Systemic exposure to bevacizumab was at the same level when administered by intravitreal or subconjunctival injection.

Conclusions. Intravitreal injection of bevacizumab was the most effective route of administration for intraocular tissue. Also, bevacizumab injected subconjunctivally was transported into the intraocular tissues of the treated eyes at an effective level. Both intravitreal and subconjunctival injections of bevacizumab resulted in high plasma concentrations. Bevacizumab was distributed into the intraocular tissues in fellow eyes via the systemic circulation. This treatment may be effective for blocking vascular endothelial growth factor activity. (*Invest Ophthalmol Vis Sci.* 2009;50: 4807–4813) DOI:10.1167/iovs.08-3148

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Bevacizumab is a full-length humanized monoclonal antibody that binds all isoforms of vascular endothelial growth factor (VEGF). Intravitreal injection of bevacizumab is effective and widely used in age-related macular degeneration to prevent the development of choroidal neovascularization,[1–3] to prevent retinal neovascularization in proliferative diabetic retinopathy,[4–6] and to treat macular edema in diabetic retinopathy,[7,8] retinal vein occlusion,[9,10] and uveitis.[11,12] In addition, intravitreal bevacizumab has been used to prevent iris neovascularization.[13,14]

The possible effects of intravitreal injection of bevacizumab on the noninjected fellow eye have been reported previously.[4,15–18] These effects may be due to the possibility that bevacizumab enters the fellow by the systemic circulation. In rabbit eyes, Bakri et al.[19] measured the pharmacokinetics of bevacizumab in the aqueous and vitreous humors in the administrative and the fellow eye as well as in the plasma after intravitreal injection and demonstrated that bevacizumab was detected in the aqueous and vitreous humors in the fellow eye and the plasma.

One area of investigation for this drug has been how well it penetrates and localizes to the retina, choroid, and iris/ciliary body after intravitreal injection. Three groups demonstrated previously that after intravitreal injection, bevacizumab penetrated the retina in the mouse eye,[20] rabbit eye,[21] and monkey eye.[22] Recently, Peters et al.[23] showed that after intravitreal injection into the primate eye, bevacizumab was located in the blood vessel walls of the iris and ciliary body. However, there are no studies in the current literature that have measured the concentration of bevacizumab in the retina/choroid and iris/ciliary body as target tissues for neovascularization.

Bevacizumab is commonly administered by intravitreal injection, but recently it has been shown to be effective when administered subconjunctivally[24–27] and topically (eye drops),[28–30] in corneal neovascularization as well as experimentally in rabbit corneal neovascularization models.[31,32] However, the pharmacokinetics of bevacizumab when administered subconjunctivally and topically has yet to be investigated until now.

In this study we investigated the pharmacokinetics of bevacizumab in the treated and fellow eyes, and in plasma in rabbits for three different routes of administration: intravitreal injection, subconjunctival injection, and eye drops. We also investigated the contribution of topical and systemic absorption in the retina/choroid and iris/ciliary body for three administration routes.

Material and Methods

Animals

Seventy-two Dutch Belted rabbits, weighing 1.9 to 2.5 kg each, were obtained from Biotek Co., Ltd. (Saga, Japan). The animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animals were divided into three

TABLE 1. Comparison of Pharmacokinetic Parameters Administered by Eye Drops and Subconjunctival or Intravitreal Injection

	Eye-Drops		SCJ		IVT	
Parameter/Route	**Treated**	**Fellow**	**Treated**	**Fellow**	**Treated**	**Fellow**
$T_{1/2}$, wk						
Iris/ciliary body	NC	NC	1.80	NC	0.82	NC
Vitreous	NC	NC	2.29	NC	0.85	NC
Retina/choroid	NC	NC	2.85	NC	0.89	NC
Plasma	NC		1.75		1.85	
C_{max}, ng/g						
Iris/ciliary body	16.1	11.7	1418.7	1192.9	109192.6	753.6
Vitreous	1.7	0.4	11.1	9.3	59730.8	6.7
Retina/choroid	18.2	10.2	295.8	187.0	93990.0	224.2
Plasma	14.3		3733.1		2087.2	
$AUC_{0\text{-last}}$/D, (ng · wk/g)/mg						
Iris/ciliary body	1.8	1.2	1905.6	1094.1	125089.7	1640.8
Vitreous	0.2	0.1	44.7	33.6	68353.0	8.0
Retina/choroid	1.9	1.3	645.1	440.5	179438.5	750.5
Plasma	1.4		8945.7		6088.9	
Effective duration, wk						
Above 22 ng/mL						
Iris/ciliary body	NC	NC	8.4	NC	10.3	NC
Retina/choroid	NC	NC	8.6	5.2	11.7	8.0
Above 500 ng/mL						
Iris/ciliary body	NC	NC	0.3	NC	6.6	NC
Retina/choroid	NC	NC	NC	NC	7.7	NC

Pharmacokinetic parameters were calculated from mean values obtained at each time point. SCJ, subconjunctival injection; IVT, intravitreal injection; D, administered dose of bevacizumab; NC, not calculated.

groups: treatment by daily topical administration of bevacizumab (group 1), a single subconjunctival injection of bevacizumab (group 2), and a single intravitreal injection of bevacizumab (group 3).

Bevacizumab Administration

In group 1, 24 rabbits received 1.25 mg/0.05 mL of bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA) topically six times per day for the first 7 days in the right eye. In group 2, 24 rabbits received a single subconjunctival injection (1.25 mg/0.05 mL) of bevacizumab into the right eye, through a syringe (Hamilton, Reno, NV) with a 30-gauge needle, 3 to 4 mm from the limbus at the 12-o'clock position. In group 3, 24 rabbits received a single intravitreal injection (1.25 mg/0.05 mL) of bevacizumab into the right eye through the same size needle. Before an intravitreal or a subconjunctival injection, group 2 and 3 rabbits were systemically anesthetized with a mixture of xylazine hydrochloride (Celactal; Bayer Medical Ltd., Leverkeusen, Germany) and ketamine hydrochloride (Ketalar; Daiichi Sankyo Co., Ltd., Tokyo, Japan), and topically anesthetized with 0.4% oxybuprocaine hydrochloride (Benoxyl; Santen Pharmaceutical Co., Ltd., Osaka, Japan).

Enzyme-Linked Immunosorbent Assay for Bevacizumab

At predetermined intervals (1, 2, 4, or 12 weeks after administration), the rabbits were killed with an overdose of sodium pentobarbital (Somnopentyl; Kyoritsu Seiyaku Co., Tokyo, Japan), the eyes enucleated, and blood samples collected. The aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid were separated. The tissue samples of iris/ciliary body, vitreous, and retina/choroid were homogenized (CellLytic MT; C3228, Sigma-Aldrich, St. Louis, MO). Plasma was obtained from the blood sample for 10 minutes by centrifugation (600*g*) at room temperature. Bevacizumab concentrations in plasma, aqueous and vitreous humors, iris/ciliary body, and retina/choroid in the treated and the fellow eyes were determined by sandwich ELISA using 1 μg/mL of rabbit anti-human IgG (H+L) (AffiniPure, Catalog no: 309-005-082; Jackson ImmunoResearch, West Grove, PA) as a primary antibody and an ELISA kit (Protein Detector; Catalog no.: 54-62-10; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). The lower limit of quantification for this method was 0.1 ng/ mL.

Calculation of Pharmacokinetic Parameters

Pharmacokinetic parameters of drugs in the vitreous, iris/ciliary body, retina/choroid, and plasma including half-life ($T_{1/2}$), C_{max}, and the area under the curve $(AUC)_{0\text{-last}}$ were calculated from mean values obtained at each time point with commercial software (WinNonlin Professional, ver. 5.2; Pharsight Co. Mountain View, CA). $T_{1/2}$ was obtained to follow first-order kinetics. Relative contribution of topical and systemic absorption in the iris/ciliary body and retina/choroid of the treated eyes when administered by three different routes were calculated by the following equations:

$$\text{Contribution of topical absorption (\%)} = 100 \cdot [(C_{max(\text{treated eyes})} - C_{max(\text{fellow eyes})})/C_{max(\text{treated eyes})}] \quad (1)$$

$$\text{Contribution of systemic absorption (\%)} = 100 - (\text{contribution of topical absorption}) \quad (2)$$

RESULTS

Ocular Pharmacokinetics

Eye Drops. The concentration of bevacizumab in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid after eye drop administration is shown in Table 1 and Figure 1. In the treated eyes (Fig. 1A), the C_{max} of bevacizumab in the iris/ciliary body and retina/choroid were 16.1 ± 2.8 and 18.2 ± 4.2 ng/g (mean ± SE) at 1 week after the start of drug administration, respectively. Topical administration was only on days 1 to 7. Two weeks after the start of topical administration, a very low level of bevacizumab was detected in the aqueous (0.6 ± 0.6 ng/ mL) and the vitreous (1.7 ± 0.3 ng/ mL) humors. In the fellow eyes (Fig. 1B), the C_{max} in the iris/ciliary body and retina/choroid were 11.7 ± 2.1 and 10.2 ± 1.2 ng/g, respectively, at 1 week after the

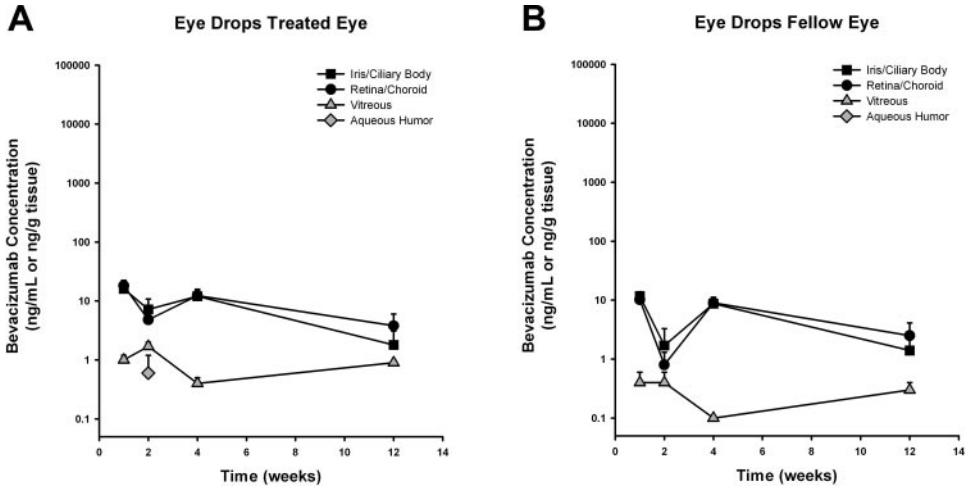

FIGURE 1. Bevacizumab concentration in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid after topical administration of bevacizumab (1.25 mg/0.05 mL; six times daily for the first 7 days) in the treated (**A**) and fellow (**B**) eyes.

start of topical drug administration. In the vitreous, a very low level of bevacizumab was detected (0.4 ± 0.2 ng/ mL, both 1 and 2 weeks after the start of topical drug administration) and bevacizumab was not detected in the aqueous.

Subconjunctival Injection. The concentrations of bevacizumab in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid after subconjunctival administration are shown in Table 1 and Figure 2. In the treated eyes (Fig. 2A), the C_{max} in the iris/ciliary body and retina/choroid was 1418.7 ± 359.2 and 295.8 ± 48.1 ng/g at 1 week after administration, respectively. The $T_{½}$ in the iris/ciliary body, and retina/choroid was 1.80 and 2.85 weeks, respectively. A low level of bevacizumab was detected in the aqueous (31.0 ± 5.2 ng/mL at 1 week after administration) and the vitreous (11.1 ± 1.0 ng/mL at 1 week) humors.

On the other hand, in the fellow eyes (Fig. 2B), the C_{max} of bevacizumab in the iris/ciliary body and retina/choroid were 1192.9 ± 80.8 and 187.0 ± 26.7 ng/g at 1 week after administration, respectively. A low level of bevacizumab was detected in the aqueous (11.9 ± 2.3 ng/mL at 1 week after administration) and the vitreous (9.3 ± 0.6 ng/mL at 2 weeks) humors.

Intravitreal Injection. The concentrations of bevacizumab in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid after intravitreal administration are shown in Table 1 and Figure 3. In the treated eyes (Fig. 3A), the C_{max} of bevacizumab in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid were 373.6 ± 150.6 ng/mL, 109,192.6 ± 13,273.1 ng/g, 59,730.8 ± 10,552.7 ng/mL, and 93,990.0 ± 38,271.6 ng/g, at 1 week after administration, respectively. The $T_{½}$ in the iris/ciliary body, and retina/choroid were 0.82 and 0.89 weeks, respectively.

In the fellow eyes (Fig. 3B), a very low level of bevacizumab was detected in the aqueous (5.4 ± 5.4 ng/mL at 1 week) and vitreous (6.7 ± 6.7 ng/mL at 2 weeks) humors. The C_{max} of bevacizumab in iris/ciliary body and retina/choroid were 753.6 ± 221.9 ng/g at 2 weeks and 224.2 ± 34.3 ng/g at 1 week after administration, respectively.

Systemic Pharmacokinetics

The concentrations of bevacizumab in the plasma from each of the three types of administration routes are shown in Table 1 and Figure 4.

In the case of eye drops, the C_{max} in the plasma was 14.3 ± 6.4 ng/mL at 1 week after administration. For subconjunctival injection, the C_{max} was 3733.1 ± 174.9 ng/mL at 1 week after injection and $T_{½}$ was 1.75 weeks. For intravitreal injection, the C_{max} was 2087.2 ± 200.8 ng/mL at 2 weeks after injection and $T_{½}$ was 1.85 weeks.

Contribution of Topical and Systemic Absorption in the Iris/Ciliary Body and Retina/Choroid of the Treated Eye

Figure 5 shows the percentage of bevacizumab concentration in the iris/ciliary body and retina/choroid delivered by topical

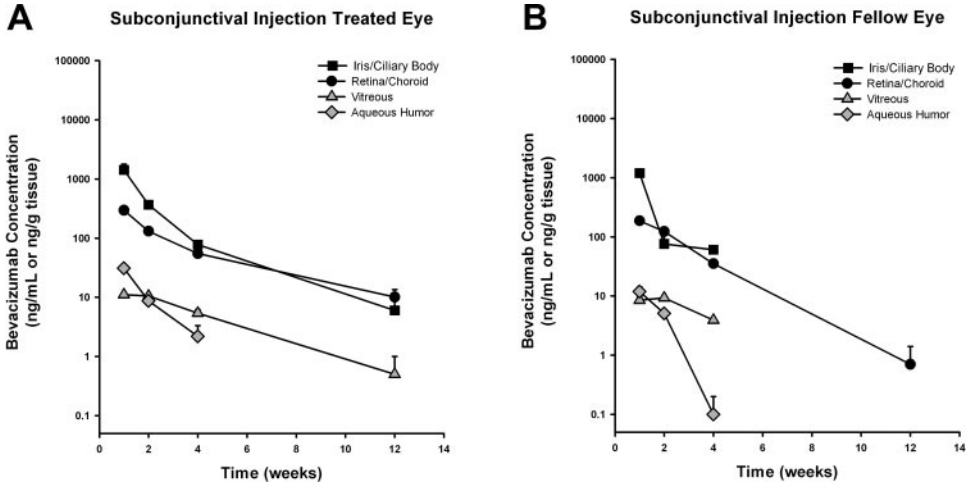

FIGURE 2. Bevacizumab concentration in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid after subconjunctival injection (1.25 mg/0.05 mL) into the treated (**A**) and fellow (**B**) eyes.

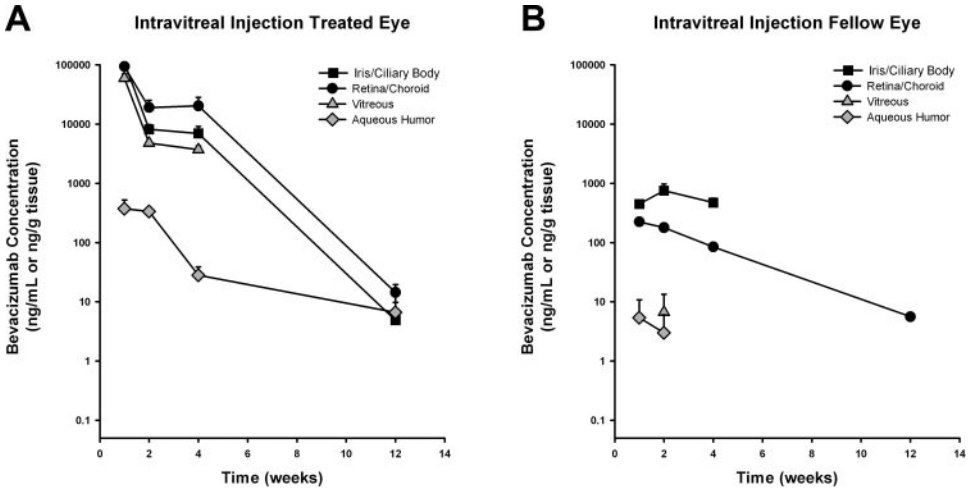

FIGURE 3. Bevacizumab concentration in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid after intravitreal injection (1.25 mg/0.05 mL) into the treated (**A**) and fellow (**B**) eyes.

and systemic absorption when administered by the three different routes. In the case of intravitreal injection, most of the bevacizumab detected in the retina/choroid and iris/ciliary body was a result of the direct injection of bevacizumab, with less than 1% obtained from the systemic circulation. For subconjunctival injection, 20% and 40% of the total absorbed bevacizumab in the iris/ciliary body and the retina/choroid, respectively, penetrated through the sclera. For the eye drops, 30% and 50% of the total absorbed bevacizumab in the iris/ciliary body and the retina/choroid, respectively, were absorbed via the transcorneal and/or transscleral route. Therefore, after subconjunctival injection or eye drops, most of the bevacizumab in the iris/ciliary body and retina/choroid was absorbed systemically.

DISCUSSION

Other studies have described the pharmacokinetics of bevacizumab after intravitreal injection of bevacizumab in rabbits[19] and humans (Csaky KG, et al. *IOVS.* 2007;48:ARVO E-Abstract 4936).[33,34] It is important to understand the pharmacokinetics of drugs administered via two additional routes because there have been reports of increased bioavailability of bevacizumab by subconjunctival injection[24–27] and topical solution.[28–30] In this study, we compared bevacizumab pharmacokinetics in ocular tissue and plasma after administration by repeated doses in eye drops, a single subconjunctival injection, or a single intravitreal injection. It should be noted that our rabbit study model is somewhat different from the human eye, as the rabbit vitreous volume is smaller, the lens is larger, and the retina is thinner, is avascular, and has no fovea. Therefore, the behavior of bevacizumab in human eyes may differ from that in rabbit eyes. Moreover, the actual therapeutic concentration of bevacizumab for the treatment of iris, retinal, and choroidal neovascularization is still unknown. Results in in vitro studies, however, have suggested that a molar ratio of 2.6 to 1 of bevacizumab to $VEGF_{165}$ is necessary for maximum inhibition of endothelial proliferation. The half-maximum inhibitory concentration of bevacizumab (IC_{50}) is 22 ng/mL, and the minimum concentration that completely blocks VEGF activity, including VEGF-induced endothelial cell growth, migration, and hyperpermeability, is 500 ng/mL.[35]

Our study showed that the most effective administration route to the retina/choroid and iris/ciliary body was intravitreal injection. Csaky et al. showed that bevacizumab elimination

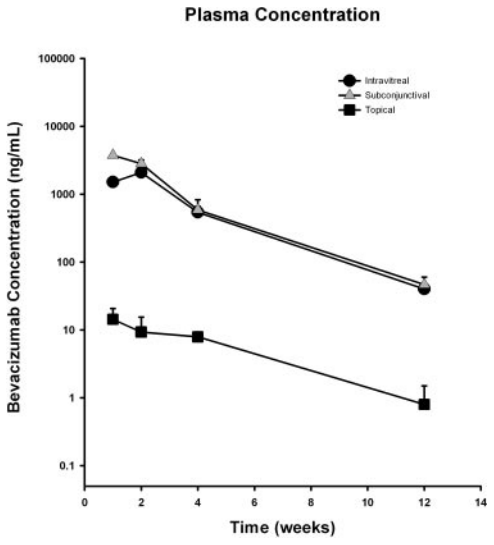

FIGURE 4. Bevacizumab concentration in the plasma after topical, subconjunctival, and intravitreal injection (1.25 mg/0.05 mL).

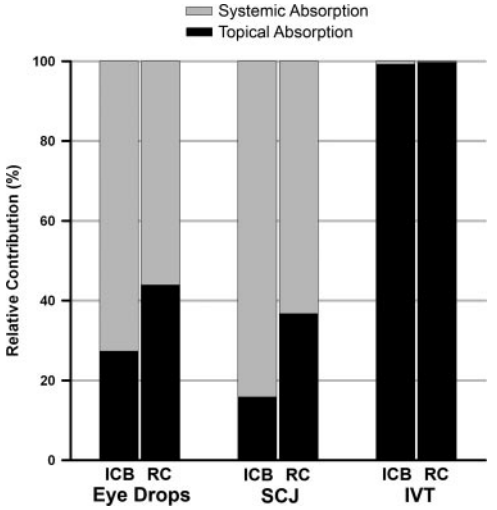

FIGURE 5. Relative contribution of topical and systemic absorption in the retina/choroid and iris/ciliary body of the treated eyes when administered by topical, subconjunctival, and intravitreal injection. ICB, iris/ciliary body; RC, retina/choroid; SCJ, subconjunctival injection; IVT, intravitreal injection.

followed first-order kinetics with $T_{1/2}$ in the human vitreous of approximately 10 days (Csaky KG, et al. *IOVS.* 2007;48:ARVO E-Abstract 4936). In contrast, Zhu et al.[33] reported that the pharmacokinetics of intravitreal bevacizumab follow a two-compartment model with initial and terminal $T_{1/2}$ of 0.5 and 6.7 days, respectively. Our half-life results in rabbits were slightly shorter ($T_{1/2} \sim 6$ days) compared with the results of Csaky et al. and Zhu et al. in humans.

In addition, Bakri et al.[19] showed in rabbits that after a 1.25-mg intravitreal injection of bevacizumab, in the treated eye, the $T_{1/2}$ in the aqueous and vitreous humors was 4.88 and 4.32 days, respectively. In contrast, in our rabbit study, $T_{1/2}$ in the vitreous after direct injection was approximately 6 days.

The differences in our results and those of these previous studies could be explained by a difference in ocular anatomy as well as detection methods for bevacizumab. Csaky et al. (*IOVS.* 2007;48:ARVO E-Abstract 4936) and Zhu et al.[33] performed their studies in humans, whereas we used rabbits. The vitreous volume of rabbits is approximately 1.5 mL, which is only one third the volume in humans. A larger vitreous volume may need a longer duration for equal distribution and therefore possibly a longer $T_{1/2}$ in the human vitreous. This notion would be in accordance with the comparison of the pharmacokinetics of triamcinolone acetonide in rabbits[36] and humans.[37]

Furthermore, although we and Bakri et al.[19] both used rabbits, the pharmacokinetic parameters in Bakri et al. differed from those in our study in that their method detected only free bevacizumab. In this study, we used the rabbit anti-human IgG (H+L) (AffiniPure; Jackson ImmunoResearch, Inc.) which has the ability to detect both the heavy and light chains of bevacizumab. Therefore, our assay had the ability to detect three possible variants of bevacizumab in vitro: fragments of the bevacizumab molecules, the entire VEGF-bevacizumab complex, and free bevacizumab.

In the retina/choroid and iris/ciliary body after intravitreal injection, bevacizumab concentration above IC_{50} was maintained for approximately 11.7 and 10.3 weeks, respectively, whereas that above 500 ng/mL was maintained for 7.7 and 6.6 weeks. A recent report regarding long-term follow-up results for intravitreal bevacizumab treatment of neovascular age-related macular degeneration has described the mean number of injections during 1 year as 3.4.[1] Thus, a single intravitreal injection of bevacizumab is active for approximately 3 months. Our results in rabbits showed bevacizumab concentration in the retina/choroid after intravitreal injection were maintained for approximately 3 months (11.7 weeks) in the effective range and were consistent with reinjection intervals in clinical situations.

Subconjunctival injection is also an effective mode of administration for intraocular neovascular diseases. In general, drug injected into subconjunctival space has two fates: direct transscleral delivery into intraocular tissues or clearance via conjunctival blood and lymphatic flow.[38–40] Since IgG has a relatively high scleral permeability and has the same molecular weight as bevacizumab, some of the bevacizumab injected into the subconjunctival space may penetrate intraocular tissues including the retina/choroid, iris/ciliary body, and vitreous via the sclera.[41,42] The bevacizumab level in the retina/choroid and iris/ciliary body was maintained above IC_{50} for 8.6 and 8.4 weeks, respectively, whereas it was maintained above 500 ng/mL for 0.3 weeks in the iris/ciliary body. The sclera consists of collagen and elastin chains that create a fiber matrix in which the pore diameter and intracellular space may determine the permeability of drugs. Negatively charged drugs have been found to have higher permeability than those with positive charges in bovine and porcine sclera.[43,44] Proteoglycans in the sclera are negatively charged,[45] which may contribute to the binding of positively charged molecules. An isoelectric point of bevacizumab has been found to be approximately 8.4.[46] Therefore, longer $T_{1/2}$ in the iris/ciliary body and retina/choroid after subconjunctival injection compared with those after intravitreal injection may sustain bevacizumab delivery into intraocular tissues due to scleral depot binding of bevacizumab to the scleral matrix.

Since the conjunctival blood vessels do not form a tight junction barrier,[47] bevacizumab can enter into the blood circulation by pinocytosis and/or convective transport through paracellular pores in the vascular endothelial layer. The Fc receptor, which binds to both albumin and the Fc portion of IgG,[48,49] was detected in the lymphatic vessels but not in the blood vessels of the conjunctiva.[50] It may be that the function of the Fc receptor in the conjunctival lymphatic vessels is to act as an efflux receptor for the efficient elimination from the conjunctival space. Residual bevacizumab, other than the bevacizumab that directly permeated the sclera and was introduced into the blood circulation, may be eliminated from the conjunctival tissue into the lymphatic vessels via convective transport with lymphatic fluid.[51]

Systemic exposure of bevacizumab when administered by intravitreal and subconjunctival injection was very similar. Bevacizumab was detected in the fellow eyes treated by intravitreal or subconjunctival injection. Bevacizumab may have been transported through the systemic circulation into the fellow eyes. In the fellow eyes treated by intravitreal injection, bevacizumab concentration in the retina/choroid was maintained above IC_{50} for 8.0 weeks.

In the case of subconjunctival injection, our data showed that most of the bevacizumab in the treated eyes was derived from the systemic circulation, and so the retinal and choroidal pharmacokinetics profile in the fellow eyes was similar to that in the treated eyes. In the fellow eyes, bevacizumab concentration in the retina/choroid was maintained above IC_{50} for 5.2 weeks. In the iris/ciliary body, a similar profile was observed, but in the fellow eye, bevacizumab was not detected at 12 weeks after injection. Of interest, corneal deposition of bevacizumab for 4 weeks after subconjunctival injection was observed by immunohistochemistry (data not shown). Therefore, subconjunctival bevacizumab may be a promising treatment, not only for neovascular diseases of the iris, retina, and choroid but also for corneal neovascularization.

In this study, we concluded that topical administration of bevacizumab by eye drops was not effective for the treatment of intraocular neovascular diseases because of poor distribution in the target tissues. These results are in accordance with data that showed that a monoclonal antibody cannot penetrate the cornea from an in vitro chamber system.[52] Since previous reports showed that topically applied bevacizumab was effective for corneal neovascularization,[28–30] further studies are needed regarding the pharmacokinetics of drugs administered to the cornea in eye drops.

There are several reports that have noted the systemic adverse effects of bevacizumab after intravitreal injection.[53–55] These adverse effects are similar to the ones reported for intravenous administration of bevacizumab for cancer treatment, such as systemic hypertension, thromboembolic diseases and death.[56,57] In addition, Shima et al.[55] found irregular vaginal bleeding as a complication in young women receiving intravitreal injection of bevacizumab. On the other hand, there have been no complications reported in previous studies of bevacizumab injected subconjunctivally at a concentration of 2.5 mg/0.1 mL,[24–27] which is twice the dose that we used in the current study. It should be noted, however, that these were small studies with a small amount of participants. Our results suggest that systemic adverse effects of bevacizumab may occur after subconjunctival injection, as well as after intravitreal or intravenous injection. Therefore, intravitreal or subconjunc-

tival bevacizumab should be used in elderly patients with choroidal or iris neovascularization. However, efficacy in the fellow eye should be expected to derive from the systemic circulation.

In conclusion, in this study we investigated the pharmacokinetics of bevacizumab in intraocular tissues and plasma by three different routes of administration. First, we found that intravitreal injection of bevacizumab was the most effective route of administration for intraocular tissue. Second, we found that both intravitreal and subconjunctival injections of bevacizumab resulted in a high concentration in plasma, and bevacizumab was distributed into the intraocular tissues in fellow eyes via systemic circulation at a level that may be effective in blocking VEGF activity. In addition, when administered by subconjunctival injection, bevacizumab was transported systemically into intraocular tissues of treated eyes at an effective level. More research is needed to define the ideal dose of bevacizumab, administration route, and intervals that will achieve the best clinical outcome possible without systemic side effects.

References

1. Bashshur ZF, Haddad ZA, Schakal A, Jaafar RF, Saab M, Noureddin BN. Intravitreal bevacizumab for treatment of neovascular age-related macular degeneration: a one-year prospective study. *Am J Ophthalmol.* 2008;145:249–256.
2. Arevalo JF, Fromow-Guerra J, Sanchez JG, et al. Primary intravitreal bevacizumab for subfoveal choroidal neovascularization in age-related macular degeneration: results of the Pan-American Collaborative Retina Study group at 12 months follow-up. *Retina.* 2008; 28:1387–1394.
3. Avila MP, Farah ME, Santos A, Duprat JP, Woodward BW, Nau J. Twelve-month short-term safety and visual acuity results from a multicentre, prospective study of epiretinal strontium-90 brachytherapy with bevacizumab for the treatment of subfoveal choroidal neovascularisation secondary to age-related macular degeneration. *Br J Ophthalmol.* 2008;93:305–309.
4. Avery RL, Pearlman J, Pieramici DJ, et al. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. *Ophthalmology.* 2006;113:1695–1705.
5. Sawada O, Kawamura H, Kakinoki M, Sawada T, Ohji M. Vascular endothelial growth factor in aqueous humor before and after intravitreal injection of bevacizumab in eyes with diabetic retinopathy. *Arch Ophthalmol.* 2007;125:1363–1366.
6. Rizzo S, Genovesi-Ebert F, Di Bartolo E, Vento A, Miniaci S, Williams G. Injection of intravitreal bevacizumab (Avastin) as a preoperative adjunct before vitrectomy surgery in the treatment of severe proliferative diabetic retinopathy (PDR). *Graefes Arch Clin Exp Ophthalmol.* 2008;246:837–842.
7. Kook D, Wolf A, Kreutzer T, et al. Long-term effect of intravitreal bevacizumab (Avastin) in patients with chronic diffuse diabetic macular edema. *Retina.* 2008;28:1053–1060.
8. Arevalo JF, Sanchez JG, Fromow-Guerra J, et al. Comparison of two doses of primary intravitreal bevacizumab (Avastin) for diffuse diabetic macular edema: results from the Pan-American Collaborative Retina Study group (PACORES) at 12-month follow-up. *Graefes Arch Clin Exp Ophthalmol.* 2009;247(6):735–43.
9. Costa RA, Jorge R, Calucci D, Melo LA Jr, Cardillo JA, Scott IU. Intravitreal bevacizumab (Avastin) for central and hemicentral retinal vein occlusions: IBeVO study. *Retina.* 2007;27:141–149.
10. Prager F, Michels S, Kriechbaum K, et al. Intravitreal bevacizumab (Avastin) for macular edema secondary to retinal vein occlusion: twelve-month results of a prospective clinical trial. *Br J Ophthalmol.* 2009;93(4):452–456.
11. Cordero Coma M, Sobrin L, Onal S, Christen W, Foster CS. Intravitreal bevacizumab for treatment of uveitic macular edema. *Ophthalmology.* 2007;114:1574–1579.
12. Ziemssen F, Deuter CM, Stuebiger N, Zierhut M. Weak transient response of chronic uveitic macular edema to intravitreal bevacizumab (Avastin). *Graefes Arch Clin Exp Ophthalmol.* 2007;245: 917–918.
13. Avery RL. Regression of retinal and iris neovascularization after intravitreal bevacizumab (Avastin) treatment. *Retina.* 2006;26: 352–354.
14. Wakabayashi T, Oshima Y, Sakaguchi H, et al. Intravitreal bevacizumab to treat iris neovascularization and neovascular glaucoma secondary to ischemic retinal diseases in 41 consecutive cases. *Ophthalmology.* 2008;115:1571–1580.
15. Mennel S, Callizo J, Schmidt JC, Meyer CH. Acute retinal pigment epithelial tear in the untreated fellow eye following repeated bevacizumab (Avastin) injections. *Acta Ophthalmol Scand.* 2007; 85:689–691.
16. Charbel Issa P, Finger RP, Holz FG, Scholl HP. Eighteen-month follow-up of intravitreal bevacizumab in type 2 idiopathic macular telangiectasia. *Br J Ophthalmol.* 2008;92:941–945.
17. Yoon YH, Kim JG, Chung H, Lee SY. Rapid progression of subclinical age-related macular degeneration in the untreated fellow eye after intravitreal bevacizumab. *Acta Ophthalmol*. Published online August 13, 2008.
18. Sawada O, Kawamura H, Kakinoki M, Ohji M. Vascular endothelial growth factor in fellow eyes of eyes injected with intravitreal bevacizumab. *Graefes Arch Clin Exp Ophthalmol.* 2008;246: 1379–1381.
19. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). *Ophthalmology.* 2007;114: 855–859.
20. Heiduschka P, Julien S, Hofmeister S, Bartz-Schmidt KU, Schraermeyer U. Bevacizumab (Avastin) does not harm retinal function after intravitreal injection as shown by electroretinography in adult mice. *Retina.* 2008;28:46–55.
21. Shahar J, Avery RL, Heilweil G, et al. Electrophysiologic and retinal penetration studies following intravitreal injection of bevacizumab (Avastin). *Retina.* 2006;26:262–269.
22. Heiduschka P, Fietz H, Hofmeister S, et al. Penetration of bevacizumab through the retina after intravitreal injection in the monkey. *Invest Ophthalmol Vis Sci.* 2007;48:2814–2823.
23. Peters S, Heiduschka P, Julien S, Bartz-Schmidt KU, Schraermeyer U. Immunohistochemical localisation of intravitreally injected bevacizumab in the anterior chamber angle, iris and ciliary body of the primate eye. *Br J Ophthalmol.* 2008;92:541–544.
24. Awadein A. Subconjunctival bevacizumab for vascularized rejected corneal grafts. *J Cataract Refract Surg.* 2007;33:1991–1993.
25. Bahar I, Kaiserman I, McAllum P, Rootman D, Slomovic A. Subconjunctival bevacizumab injection for corneal neovascularization. *Cornea.* 2008;27:142–147.
26. Bahar I, Kaiserman I, McAllum P, Rootman D, Slomovic A. Subconjunctival bevacizumab injection for corneal neovascularization in recurrent pterygium. *Curr Eye Res.* 2008;33:23–28.
27. Erdurmus M, Totan Y. Subconjunctival bevacizumab for corneal neovascularization. *Graefes Arch Clin Exp Ophthalmol.* 2007;245: 1577–1579.
28. Kim SW, Ha BJ, Kim EK, Tchah H, Kim TI. The effect of topical bevacizumab on corneal neovascularization. *Ophthalmology.* 2008;115:33–38.
29. Bock F, Konig Y, Kruse F, Baier M, Cursiefen C. Bevacizumab (Avastin) eye drops inhibit corneal neovascularization. *Graefes Arch Clin Exp Ophthalmol.* 2008;246:281–284.
30. Uy HS, Chan PS, Ang RE. Topical bevacizumab and ocular surface neovascularization in patients with Stevens-Johnson syndrome. *Cornea.* 2008;27:70–73.
31. Papathanassiou M, Theodossiadis PG, Liarakos VS, Rouvas A, Giamarellos-Bourboulis EJ, Vergados IA. Inhibition of corneal neovascularization by subconjunctival bevacizumab in an animal model. *Am J Ophthalmol.* 2008;145:424–431.
32. Kim TI, Kim SW, Kim S, Kim T, Kim EK. Inhibition of experimental corneal neovascularization by using subconjunctival injection of bevacizumab (Avastin). *Cornea.* 2008;27:349–352.
33. Zhu Q, Ziemssen F, Henke-Fahle S, et al. Vitreous levels of bevacizumab and vascular endothelial growth factor-A in patients with choroidal neovascularization. *Ophthalmology.* 2008;115:1750–1755; 1755 e1751.
34. Krohne TU, Eter N, Holz FG, Meyer CH. Intraocular pharmacokinetics of bevacizumab after a single intravitreal injection in humans. *Am J Ophthalmol.* 2008;146:508–512.

35. Wang Y, Fei D, Vanderlaan M, Song A. Biological activity of bevacizumab, a humanized anti-VEGF antibody in vitro. *Angiogenesis.* 2004;7:335–345.
36. Chin HS, Park TS, Moon YS, Oh JH. Difference in clearance of intravitreal triamcinolone acetonide between vitrectomized and nonvitrectomized eyes. *Retina.* 2005;25:556–560.
37. Beer PM, Bakri SJ, Singh RJ, Liu W, Peters GB 3rd, Miller M. Intraocular concentration and pharmacokinetics of triamcinolone acetonide after a single intravitreal injection. *Ophthalmology.* 2003;110:681–686.
38. Robinson MR, Lee SS, Kim H, et al. A rabbit model for assessing the ocular barriers to the transscleral delivery of triamcinolone acetonide. *Exp Eye Res.* 2006;82:479–487.
39. Ranta VP, Urtti A. Transscleral drug delivery to the posterior eye: prospects of pharmacokinetic modeling. *Adv Drug Deliv Rev.* 2006;58:1164–1181.
40. Amrite AC, Edelhauser HF, Kompella UB. Modeling of corneal and retinal pharmacokinetics after periocular drug administration. *Invest Ophthalmol Vis Sci.* 2008;49:320–332.
41. Ambati J, Canakis CS, Miller JW, et al. Diffusion of high molecular weight compounds through sclera. *Invest Ophthalmol Vis Sci.* 2000;41:1181–1185.
42. Ambati J, Gragoudas ES, Miller JW, et al. Transscleral delivery of bioactive protein to the choroid and retina. *Invest Ophthalmol Vis Sci.* 2000;41:1186–1191.
43. Maurice DM, Polgar J. Diffusion across the sclera. *Exp Eye Res.* 1977;25:577–582.
44. Cheruvu NP, Kompella UB. Bovine and porcine transscleral solute transport: influence of lipophilicity and the choroid-Bruch's layer. *Invest Ophthalmol Vis Sci.* 2006;47:4513–4522.
45. Dunlevy JR, Rada JA. Interaction of lumican with aggrecan in the aging human sclera. *Invest Ophthalmol Vis Sci.* 2004;45:3849–3856.
46. Vlckova M, Kalman F, Schwarz MA. Pharmaceutical applications of isoelectric focusing on microchip with imaged UV detection. *J Chromatogr A.* 2008;1181:145–152.
47. Raviola G. Conjunctival and episcleral blood vessels are permeable to blood-borne horseradish peroxidase. *Invest Ophthalmol Vis Sci.* 1983;24:725–736.
48. Wani MA, Haynes LD, Kim J, et al. Familial hypercatabolic hypoproteinemia caused by deficiency of the neonatal Fc receptor, FcRn, due to a mutant beta2-microglobulin gene. *Proc Natl Acad Sci U S A.* 2006;103:5084–5089.
49. Burmeister WP, Huber AH, Bjorkman PJ. Crystal structure of the complex of rat neonatal Fc receptor with Fc. *Nature.* 1994;372:379–383.
50. Kim H, Fariss RN, Zhang C, Robinson SB, Thill M, Csaky KG. Mapping of the neonatal Fc receptor in the rodent eye. *Invest Ophthalmol Vis Sci.* 2008;49:2025–2029.
51. Lobo ED, Hansen RJ, Balthasar JP. Antibody pharmacokinetics and pharmacodynamics. *J Pharm Sci.* 2004;93:2645–2668.
52. Brereton HM, Taylor SD, Farrall A, et al. Influence of format on in vitro penetration of antibody fragments through porcine cornea. *Br J Ophthalmol.* 2005;89:1205–1209.
53. Fung AE, Rosenfeld PJ, Reichel E. The International Intravitreal Bevacizumab Safety Survey: using the internet to assess drug safety worldwide. *Br J Ophthalmol.* 2006;90:1344–1349.
54. Wu L, Martinez-Castellanos MA, Quiroz-Mercado H, et al. Twelve-month safety of intravitreal injections of bevacizumab (Avastin®): results of the Pan-American Collaborative Retina Study group (PACORES). *Graefes Arch Clin Exp Ophthalmol.* 2008;246:81–87.
55. Shima C, Sakaguchi H, Gomi F, et al. Complications in patients after intravitreal injection of bevacizumab. *Acta Ophthalmol.* 2008;86:372–376.
56. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med.* 2004;350:2335–2342.
57. Ratner M. Genentech discloses safety concerns over Avastin. *Nat Biotechnol.* 2004;22:1198.