Pharmacokinetics of Bevacizumab and Its Effect on Vascular Endothelial Growth Factor after Intravitreal Injection of Bevacizumab in Macaque Eyes

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PURPOSE. To evaluate the pharmacokinetics of intravitreally injected bevacizumab in the systemic circulation and the aqueous humor and its effect on vascular endothelial growth factor (VEGF) in the aqueous humor.

METHODS. Bevacizumab (1.25 mg/50 μL) was injected into the vitreous cavity of the right eyes of three cynomolgus macaques. Aqueous humor and serum were obtained from the macaques just before injection and on days 1, 3, 7, and 2, 4, 6, and 8 after injection. The bevacizumab and VEGF concentrations were measured using enzyme-linked immunosorbent assay.

RESULTS. Aqueous VEGF concentrations ranged from 63.2 to 106 pg/mL (mean, 80.0 ± 22.6 pg/mL) before injection; decreased to <31.2 pg/mL, the lower limit of detection, in all eyes between 1 and 28 days after injection; and returned to the preinjection concentration at 42 days. Aqueous VEGF concentrations in the fellow eyes did not change throughout the experiment. Aqueous bevacizumab concentrations in the treated eyes reached a mean peak concentration of 49,500 ± 10,900 ng/mL the day after injection and gradually declined, whereas those in the untreated eyes peaked at 3 days, with a mean concentration of 18.5 ± 25.5 ng/mL, and declined to below 0.156 ng/mL, the limit of detection at 2 weeks. A maximum mean bevacizumab concentration of 1450 ± 186 ng/mL was achieved in the serum 1 week after injection.

CONCLUSIONS. Intravitreal injection of bevacizumab decreased the VEGF concentration in the treated eyes for at least 4 weeks and had no or a minimal effect on the untreated fellow eyes.

Bevacizumab (Avastin; Genentech, South San Francisco, CA) is a full-length humanized monoclonal antibody to all isoforms of vascular endothelial growth factor (VEGF) and has been approved by the Food and Drug Administration for intravenous treatment of metastatic colorectal cancer. Recently, intravenous injection of bevacizumab was reported to be effective for treating age-related macular degeneration (AMD), whereas intravitreal injection of bevacizumab has been widely used to treat various ocular diseases including AMD and proliferative diabetic retinopathy. Although numerous reports about the efficacy of intravitreal injection of bevacizumab have been published, few studies have reported on the pharmacokinetics of bevacizumab. Bakri et al.6 reported the pharmacokinetics of intravitreal bevacizumab in a rabbit model and clearly showed that the vitreous half-life of 1.25 mg intravitreal bevacizumab is 4.52 days in rabbit eyes, with minute amounts of bevacizumab detected in the serum and the fellow untreated eye. However, the study had some limitations because of differences in vitreous volume and anatomy of human eyes. Therefore, we used a primate model, which has several advantages in that the ocular volume and anatomy are similar to those of humans. We measured the VEGF and bevacizumab concentrations over time in the aqueous humor of the treated and the untreated eyes after intravitreal injection of bevacizumab in cynomolgus macaques and the pharmacokinetics of bevacizumab in the aqueous humor of the treated and untreated eyes and in the serum.

METHODS

All treatments were conducted in agreement with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the animal research was approved by the Animal Experimentation Committee at Shiga University of Medical Science. Three male cynomolgus macaques, aged 8 to 9 years and weighing 3.9 to 5.5 kg, were anesthetized with 5 mg/kg intramuscular ketamine hydrochloride and 1 mg/kg intramuscular xylazine hydrochloride. Povidone iodine was placed on the conjunctiva of each eye. With the use of a 29-gauge needle, bevacizumab (1.25 mg/50 μL) was injected into the vitreous cavity of the right eye of each macaque. The left eyes received no intravitreal injections and served as controls. Both aqueous humor samples (200 μL) and venous blood samples (2 mL) were obtained from the macaque just before injection and 1, 3, and 7 days and 2, 4, 6, and 8 weeks after injection. Aqueous humor samples were obtained with a 29-gauge syringe. Anterior chamber depth recovered at all times when the samples were obtained. Serum was obtained by allowing the blood sample to clot overnight at 4°C followed by centrifugation. Samples were stored in a freezer at −80°C until analysis. The eyes were monitored before injection and 1, 3, and 7 days and then weekly after injection for signs of inflammation.

Measurement of VEGF

VEGF concentrations in the aqueous humor and the serum were measured with a commercial immunoassay (Quantikine Human VEGF Immunoassay; R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. The limit of the detectable VEGF concentration was 31.2 pg/mL. We measured serum VEGF concentrations twice. However, we measured VEGF concentrations in the aqueous humor once because the sample volumes were small.

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Bevacizumab Immunoassay

The concentration of bevacizumab was measured using an enzyme-linked immunosorbent assay, as previously described with slight modification. Ninety-six-well plates were coated with recombinant human VEGFα/5 (R&D Systems) at a concentration of 1 µg/mL overnight at 4°C (100 µL/well). After washing three times with phosphate-buffered saline (PBS) containing 0.05% Tween-20, the wells were blocked with 3% bovine serum albumin/PBS overnight at 4°C (200 µL/well). The wells then were washed five times with PBS containing 0.05% Tween-20 and stored dry at 4°C for later use. Aqueous humor or serum diluted in 0.1% bovine serum albumin/PBS was added to the plates overnight at 4°C (50 µL/well). Bevacizumab was detected by horseradish peroxidase-goat anti-human IgG (H+L) conjugate (Invitrogen Corporation, Carlsbad, CA) with a concentration of 1 µg/mL after a 3-hour incubation at room temperature. After five washes, color development was performed with 100-µL tetramethyl benzidine substrates (3,3’,5,5’-tetramethyl benzidine substrate), and the reaction was stopped by the addition of 1 M hydrogen chloride (100 µL). Optical density was measured at 450 nm with correction at 570 nm. A standard curve was prepared, with bevacizumab ranging from 15.6 to 1000 pg/mL, with a regression analysis of the data using GraphPad Prism (GraphPad Software, Inc., San Diego, CA). Bevacizumab in the aqueous humor and serum was measured by the same method. The detectable limit of the immunoassay was 0.156 ng/mL. The limit of detection of the assay was 31.2 pg/mL.

Statistical Analysis

All statistical analyses were carried out with a statistical analysis program (SAS 9.1.3; SAS Institute Japan, Tokyo, Japan).

RESULTS

VEGF concentrations in the aqueous humor of the right eyes ranged from 63.2 to 106 pg/mL (mean ± SD, 80.0 ± 22.6 pg/mL) before intravitreal injection of bevacizumab. One day after injection of bevacizumab, the VEGF concentrations in the aqueous humor decreased to <31.2 pg/mL, the lower limit of detection, in all treated eyes. The concentration below the lower limit was maintained until 4 weeks in all eyes (Fig. 1). VEGF concentrations in the aqueous humor of the fellow untreated eyes (left eyes) ranged from 57.9 to 108 pg/mL (mean, 89.4 ± 27.5 pg/mL) before intravitreal injection. There were no significant differences between the treated and the untreated eyes before intravitreal injection. VEGF concentrations in the aqueous humor of the fellow eyes did not change (Fig. 2). VEGF concentrations in the serum were <31.2 pg/mL, the limit of detection, before intravitreal injection of bevacizumab throughout the experiment.

Changes in the concentration of bevacizumab over time in the aqueous humor of the treated and the untreated eyes and in the serum after intravitreal injection are shown in Figure 3. Bevacizumab concentrations in the aqueous humor of the treated eyes peaked at 49,500 ± 10,900 ng/mL the day after injection and gradually declined. Bevacizumab also was detected in the untreated eyes; however, the levels were very low. Concentrations of bevacizumab in the aqueous humor were much lower than in the aqueous humor for 1 to 2 weeks after intravitreal injection. A maximum concentration of 1430 ± 186 ng/mL was achieved 1 week after injection and then gradually declined. However, the reduction rate was lower than that in the aqueous humor of the treated eyes, and the bevacizumab concentration in the serum was higher than that in the aqueous humor in the treated eyes at 4 weeks and thereafter. The bevacizumab concentration in the serum 8 weeks after injection was 67.1 ± 24.3 ng/mL, which was approximately 187 times higher than that in the aqueous humor of the treated eyes. The half-life of 1.25 mg intravitreally injected bevacizumab was 2.8 ± 0.6 days (n = 3; range, 2.3–3.5 days) in the aqueous humor and 12.3 ± 2.6 days (n = 3; range, 9.2–14.1 days) in the serum. The area under curve was 5680 ± 2356 (µg/mL x h) in the aqueous humor and 526.2 ± 17.1 (µg/mL x h) in the serum. No complications, such as uveitis or endophthalmitis, developed after the bevacizumab injections.
DISCUSSION

Because we used a macaque model and obtained aqueous humor samples repeatedly over time, we observed the VEGF levels at different time points in the same macaque eyes. To our best knowledge, this is the first study to report the time course of the VEGF level in the same macaques. Although macaque eyes are not the same as human eyes, VEGF levels in the aqueous humor of the macaques before injection were similar to those in human eyes.5-7 Concentrations of bevacizumab in macaques also were similar to those in humans.7-8 Therefore, the current results could be applicable to human eyes. The only difference in the bevacizumab concentrations between macaques and humans was that the drug decreased in concentration in a shorter time in macaques than in humans. Krohne et al.8 reported that the half-life of an intravitreal injection of 1.5 mg bevacizumab in humans was 9.82 days in the aqueous humor. However, in the present study, the half-life of 1.25 mg bevacizumab was 3.1 days in the aqueous humor. There are several explanations for this difference. First, we observed the bevacizumab concentrations at different time points in the same macaque eyes, whereas the same patients were not observed in the clinical study. Second, we used naïve macaques in the present study, whereas the patients in the clinical study had some diseases. Measuring the VEGF and bevacizumab concentrations in the vitreous cavity rather than in the aqueous humor seems to be better for evaluating the intraocular concentration or the pharmacokinetics; however, it would be almost impossible to obtain vitreous samples from the same eyes repeatedly. Therefore, we measured VEGF and bevacizumab concentrations in the aqueous humor. The concentration in the aqueous humor can be useful because the VEGF level in the aqueous humor was reported to be significantly correlated with the VEGF level in the vitreous.9 Funatsu et al.10 measured VEGF and interleukin (IL)-6 levels in the aqueous humor, vitreous fluid, and plasma and reported a significant relationship between VEGF and IL-6 levels in the aqueous humor and vitreous fluid. The VEGF level in the vitreous fluid was about five to six times higher than in the aqueous humor. Because we clearly showed that the VEGF concentration in the aqueous humor decreased substantially after intravitreal injection of bevacizumab, the VEGF concentration in the vitreous also should decrease substantially after intravitreal injection of bevacizumab. In the present study, the VEGF level in the aqueous humor fell below the lower limit of detection after bevacizumab injection, similar to results reported in humans.3 The decreased concentration was maintained for approximately 4 weeks and returned to a level similar to that before injection at 6 weeks after injection. Therefore, the effect of intravitreal injection of bevacizumab is expected to continue for approximately 1 month in macaques; although we do not know the exact length of time, the intravitreal injection of bevacizumab continued to be effective for at least 1 month in humans.11

Aqueous humor concentrations of bevacizumab gradually declined; however, low bevacizumab concentrations were detected over 8 weeks after the intravitreal injection, and the time course of the decreasing concentration in humans is longer than in macaques, indicating that the effect might continue longer in humans.7-8 We previously reported that intravitreal injection of bevacizumab did not decrease the VEGF level in the aqueous humor of the fellow eyes and did not have as great a beneficial effect as a direct intravitreal injection of bevacizumab.12 However, because that was a clinical study, we could not measure the VEGF concentration in the aqueous humor of the untreated fellow eyes before intravitreal injection of bevacizumab in the treated eye. Therefore, we could not measure the exact decrease in those eyes.

In the present study, the VEGF concentrations in the aqueous humor of the fellow eyes did not change throughout the experiments, although a minute amount of bevacizumab was detected in the fellow untreated eyes and peaked at 3 days with a concentration of 18.5 ng/mL. Avery et al.3 reported that intravitreal injection of 6200 ng bevacizumab decreased fluorescein leakage in some cases. Because the vitreous volume is approximately 4 mL, an intravitreal injection of 6200 ng bevacizumab results in approximately 1500 ng/mL in the vitreous fluid. According to a previous study, the VEGF level in the vitreous fluid was approximately five to six times higher than in the aqueous humor.10 Therefore, 1500 ng/mL in vitreous is at least >250 ng/mL in the aqueous humor. However, in the present study, only 18.5 ng/mL was detected, and it might have been too small to have an effect. Bevacizumab was detected in the serum after intravitreal injection, though the concentrations were much lower than in the aqueous humor until 2 weeks after injection. Intravenous injection of bevacizumab 2 mg/kg once weekly in macaques was not toxic after 26 weeks, and bevacizumab concentrations in the serum 1 week after one intravenous injection of 2 mg/mL bevacizumab were higher than 10,000 ng/mL (interview form for bevacizumab, Chugai Oncology, Tokyo, Japan; available only in Japanese). The maximum concentration in serum was 1430 ± 180 ng/mL, which is much lower than 10,000 ng/mL. Therefore 1.25 mg intravitreal injections of bevacizumab are not toxic systemically.

In conclusion, intravitreal injection of bevacizumab decreased the VEGF concentration in the treated eyes for approximately 4 weeks but had no or a minimal effect on the untreated fellow eyes in macaques.

References


