Preclinical Efficacy and Safety of VEGF-Grab, a Novel Anti-VEGF Drug, and Its Comparison to Aflibercept

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Purpose. VEGF-Grab is a novel anti-vascular endothelial growth factor (VEGF) candidate drug with higher affinity to both VEGF and placental growth factor (PlGF) compared to aflibercept. We investigated the preclinical efficacy of VEGF-Grab for ophthalmic therapy and compared it to that of aflibercept.

Methods. The in vitro anti-VEGF efficacy of VEGF-Grab was determined using VEGF-induced cell proliferation/migration and tube formation assays. The in vivo antiangiogenic efficacy of intravitreal injection of either VEGF-Grab or aflibercept was evaluated using murine models of ocular angiogenesis: mouse oxygen-induced retinopathy (OIR) and rat laser-induced choroidal neovascularization (CNV). The in vivo retinal toxicity in the mouse eye resulting from the injection of either drug was evaluated with light and electron microscopy.

Results. VEGF-Grab showed greater inhibition of VEGF-induced cell proliferation/migration than aflibercept, but it showed comparable inhibition of tube formation in vitro. In the in vivo OIR model, VEGF-Grab showed a comparable suppression of retinal neovascularization compared to aflibercept. Additionally, VEGF-Grab showed an efficacy similar to that of aflibercept in terms of CNV inhibition in the laser-induced CNV model. Histology and transmission electron microscopy showed no significant signs of toxicity in the mouse retina at 7 and 30 days following the intravitreal injection of VEGF-Grab or aflibercept.

Conclusions. Compared to aflibercept, VEGF-Grab presented comparable in vivo antiangiogenic efficacy and superior in vitro anti-VEGF activity. The retinal safety profiles were comparable for the two drugs. Considering its known higher binding affinity to VEGF and PlGF compared to aflibercept, VEGF-Grab could be a potential candidate drug for neovascular retinal diseases and an alternative to aflibercept.

Keywords: anti-VEGF agent, aflibercept, retinal neovascularization, retinal safety
VEGFR1 is a receptor for multiple proangiogenic ligands, including VEGF-A, VEGF-B, and PlGF. The binding affinity of VEGFR1 to VEGF and PlGF is known to be superior to that of VEGFR2. Nevertheless, its potential as the backbone of a new decoy receptor fusion protein for therapeutic purposes has been undervalued. This is a result of its abundant positively charged residues in the third domain (D3), which lower its therapeutic efficiency by nonspecific binding to the extracellular matrix (ECM). A study found that VEGF Trap-Eye, or aflibercept, decreased its net charge by combining VEGFR1 D2 and VEGFR2 D3, leading to fewer ECM bindings and an improved pharmacokinetic profile compared to the VEGFR1 D2–D3–Fc compound molecule; however, as VEGFR2 D3 is used instead of VEGFR1 D3 by aflibercept, the high binding affinity of VEGFR1 to VEGFA and PlGF was disturbed.

To compensate this limitation related to aflibercept, a novel antiangiogenic VEGF-Grab was developed using only VEGFR1 as the backbone. This minimized the nonspecific binding to ECM while maintaining the maximum affinity to VEGF-A, VEGF-B, and PlGF through the glycosylation strategy. The molecular structure and antiangiogenic mechanisms of VEGF is described in our previous study. Early studies suggest that VEGF-Grab has a stronger and more durable antiangiogenic, antitumor, and antimetastatic efficacy than VEGF-Trap in both implanted and spontaneous tumor models; however, their toxicity profiles are comparable.

In the present paper, we propose that VEGF-Grab, a novel anti-VEGF molecule, could serve as a potential therapeutic agent for VEGF-related retinal diseases, including age-related macular degeneration, macular edema, and diabetic retinopathy. Specifically, we explored in vitro VEGF-A suppression assays, in vivo antiangiogenic efficacy, and in vivo retinal safety of VEGF-Grab.

METHODS

Approval for animal experimentation was obtained from the Seoul National University Bundang Hospital Institutional Animal Care and Use Committee (BA1706-225/052-01). Furthermore, all of the procedures for animal experiments adhered to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. VEGF-Grab was prepared in our laboratory, as previously described in the literature. The commercial anti-VEGF antibody aflibercept (Eylea, 2 mg/0.05 mL) was purchased from Bayer Pharmaceuticals (Berlin, Germany). Finally, both VEGF-Grab and aflibercept were dialisylzed with the same solvent (PBS solution) in this study.

Proliferation/Migration Assay and Tube Formation Assay

Human umbilical vein endothelial cells (HUVECs, CC-2519; Lonza Group, Basel, Switzerland), which were tested and authenticated according to the ATCC guidelines, were purchased and cultured for less than 6 months. With regard to the migration assay, as soon as the seeded HUVECs placed on the Culture-Insert 2 Well in μ-Dish (81176; Ibidi GmbH, Gräfelfing, Germany) became confluent, the culture inserts were removed to generate a wound gap. Subsequently, the cells that migrated within the wound were monitored for 24 hours in the presence of VEGF-A (5 nmol/L) with PBS solution, VEGF-Grab (25 nmol/L), or aflibercept (25 nmol/L) in EBM-2 (22011, PromoCell, Heidelberg, Germany).

Additional cellular proliferation/migration assays were conducted with serial monitoring using the IncuCyte Zoom system and software (Essen BioScience, Inc., Ann Arbor, MI, USA). As soon as the seeded HUVECs placed on the ImageLock 96-Well Plates (4806; Sartorius GmbH, Goettingen, Germany) became confluent, the Essent BioScience 96-Well WoundMaker instrument was removed to generate a wound gap. Subsequently, the cells that migrated within the wound were monitored for 24 hours in the presence of VEGF-A (5 nmol/L) and either VEGF-Grab (300 nmol/L) or aflibercept (300 nmol/L). The cell proliferation inhibitor mitomycin C (40 μg/mL, Sigma-Aldrich, St. Louis, MO, USA) in EBM-2 without supplement was added to compare VEGF-Grab and aflibercept in a proliferation-inhibited environment. The migration rate was measured at each time point as wound confluence (%).

Similarly, for the tube formation assay, HUVECs were seeded (5 × 10⁵ cells/well) on 24-well plates coated with Matrigel matrix (4 mg, 354230; Corning Inc., Corning, NY, USA) and treated with PBS solution, VEGF-Grab (25 nmol/L), or aflibercept (25 nmol/L). Finally, VEGF-A (1 nmol/L) was added 10 minutes later, and tube formations were monitored for 6 hours.

Comparison of the In Vivo Antiangiogenic Efficacy of VEGF-Grab and Aflibercept

We compared the in vivo antiangiogenic efficacy of VEGF-Grab and aflibercept in both the laser-induced choroidal neovascularization (CNV) model and the oxygen-induced retinopathy (OIR) model. The in vivo antiangiogenic efficacy of VEGF-Grab was compared with that of aflibercept in the rat CNV model and the mouse OIR model. The procedures for murine models of OIR and laser-induced CNV used below have been previously described elsewhere.

Regarding the CNV model, 36 male Brown Norway rats (body weight, 180–200 g) were housed under a normal 12 hour/12 hour light/dark cycle. For all of the animal procedures described below, animals were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg). Tropicamide (1%) was applied topically for dilation, and laser photocoagulation was performed using an argon laser (Lumenis, Inc., Santa Clara, CA, USA) with a center wavelength of 532 nm on the right eye. Incident power of 100 mW, 100 μm, and 100 ms was used to break the Bruch’s membrane, inducing four to five lesions located 0.7 mm away from the optic nerve head. Thereafter, the success of the operation was confirmed by the formation of a bubble immediately after laser photocogulation. Eyes showing significant subretinal hemorrhage after laser photocogulation were excluded from the analysis. In addition, subsequently following laser photocogulation, the rats were randomly divided into three groups to receive intravitreal injections to the right eye of one of the following: (1) aflibercept (25 mg/mL in 1 μL), (2) VEGF-Grab (25 mg/mL in 1 μL), or (3) 1 μL of PBS vehicle. All groups were sacrificed 14 days after intravitreal injection.

For the OIR model, 18-day-pregnant C57BL/6 female mice were housed under a normal 12 hour/12 hour light/dark cycle. Subsequently, neonates were exposed to 75% oxygen in a hyperoxia chamber from postnatal days 7 to 12. At post-
natal day 12 (P12), 36 mice pups were randomly divided into three groups that received aflibercept (25 mg/mL in 1 μL), VEGF-Grab (25 mg/mL in 1 μL), or 1 μL of PBS vehicle (n = 12 for each group). Aflibercept, VEGF-Grab, and the vehicle were injected intravitreally to the right eye of the animals. All groups were sacrificed 5 days after intravitreal injection (P17), which is known to be the time of maximum neovascular response.22

Preparation of Retina/Choroid Flat Mounts and Evaluation of Antiangiogenic Efficacy

All of the animals were enucleated immediately after sacrifice, which was followed by PBS perfusion. Within 4 hours after enucleation, all eyes were fixed in 2% paraformaldehyde/PBS for 10 minutes. Successively, the retina and choroid were isolated for the OIR and CNV models, respectively, and permeabilized with 0.5% Triton X-100, 5% fetal bovine serum (FBS), 5% normal goat serum, and 20% dimethylsulfoxide in PBS for 3 hours at room temperature. The retina and choroid were then incubated in a 1-μg/mL hamster anti-mouse CD31 monoclonal antibody solution at 48°C for 2 days. After washing, the retina and choroid were incubated for 4 hours at room temperature with a secondary antibody solution, a 1:300 dilution of Alexa Fluor 488 AffiniPure Goat Anti-Armenian Hamster IgG (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA, USA). Thereafter, they were incubated in a 10-μg/mL lectin BS-1-fluorescein isothiocyanate solution (Sigma-Aldrich) at 48°C for 2 days. Furthermore, four cuts were made from the edges to the center after washing the retina and choroid, which were then flattened and mounted with the vitreous edges to the center after washing the retina and choroid, which were then flattened and mounted with the vitreous side pointing upward on a microscope slide. These were then visualized with a confocal microscope (LSM 710; Carl Zeiss Meditec, Oberkochen, Germany). Finally, for quantification of antiangiogenic efficacy in the OIR model, the vascular tuft area was divided by the number of pixels in the total retinal area using ImageJ (National Institutes of Health, Bethesda, MD, USA). Thereafter, the area of retinal neovascularization was determined. Neovascularization (NV) suppression (%), as a primary outcome, was defined as (neovascular area as vascular tuft. μm²) divided by that of the contralateral eye (%). Similarly, the areas of the CNV lesions in the laser-induced CNV model were also measured using ImageJ, and the antiangiogenic efficacy was quantified.

Comparison of In Vivo Retinal Toxicity of VEGF-Grab and Aflibercept

The in vivo retinal safety of VEGF-Grab was tested and compared with that of aflibercept in a normal mouse model. Specifically, vehicle (PBS solution), VEGF-Grab (25 mg/mL in 1 μL), or aflibercept (25 mg/mL in 1 μL) was injected intravitreally into normal C57BL/6 male mice at 8 weeks. Subsequently, mice were sacrificed at 7 and 30 days following the injection, and their eyes were enucleated.

For light microscopy, a TUNEL assay using the NeuroTACS II In Situ Apoptosis Detection Kit ( Trevigen, Gaithersburg, MD, USA) was conducted. All of the eyes were sectioned in half and fixed at 4°C in a mixture of 4% paraformaldehyde in 0.1-M phosphate buffer at pH 7.4. The specimens were then stained with hematoxylin and eosin and examined with an Axiosmager A1 microscope (Carl Zeiss Meditec). Finally, samples were obtained from two different areas of all eyes in three serial sections: 500 μm inferior to the optic nerve and 4 mm from the optic nerve in the temporal–inferior quadrant.

In contrast, for transmission electron microscopy (TEM), the anterior segment was removed after enucleation, whereas the posterior cup was fixed with 2.5% glutaraldehyde in a 0.1-M sodium cacodylate buffer, pH 7.4, for 2 hours at room temperature and overnight at 4°C. Samples were post-fixed with 2% osmium tetroxide for 2 hours at room temperature, gradually dehydrated by a 10-minute exposure in an ethanol series, and finally embedded in Epon (Electron Microscopy Sciences, Hatfield, PA, USA). Eyes from both groups were compared to normal eyes without injection, as well as eyes treated with vehicle, through a qualitative analysis of the retinal layers.

Statistical Analyses

All of the results are expressed as mean ± standard deviation (SD). Statistical analyses were performed using the Mann–Whitney U test, linear regression analysis, and analysis of covariance (ANCOVA). Differences between the means were considered statistically significant for P < 0.05. The numbers of biological and experimental replicates were as follows: cell migration assay, three experiments with two wells for each group (n = 6 for each group); tube formation assay, three experiments with one or two wells for each group (n = 4 for each group); serial cell migration assay (live cell imaging using the Incucyte zoom system), three experimental replicates and 4 to 8 wells for each group (n = 12 to 24 for each group); rat OIR model experiments, one experiment using 12 rats for each group; rat CNV model experiments, three experiments with three or four mice in each group, with total CNV lesions of 52, 36, and 50 for control, VEGF-grab, and Aflibercept, respectively; and comparing in vivo retinal toxicity between VEGF-Grab and aflibercept, two experiments, two or three mice for each group.

Results

In a previous study, the inhibition of VEGFR2 signaling by both VEGF-Grab and VEGF-Trap in HUVECs was examined with the theoretical rationale that VEGF-A promotes proliferation, migration, and survival of endothelial cells through VEGFR2 activation.24 Indeed, both VEGF-Grab and aflibercept strongly suppressed VEGF-A-induced cell proliferation and migration in the in vitro assay when the VEGF-A-only medium was compared to either the VEGF-A plus VEGF-Grab or aflibercept (both P < 0.01, Mann–Whitney U test) (Fig. 1A). Furthermore, the suppression was greater with VEGF-Grab than with aflibercept (P < 0.05, Mann–Whitney U test) (Fig. 1A). In the additional serial assay, the rate of cell proliferation/migration was significantly lower for VEGF-Grab than aflibercept, confirming the superior inhibitory effect of VEGF-Grab (regression coefficient, 23.4%/min in the VEGF-Grab vs. 50.2%/min in the aflibercept; P < 0.001, ANCOVA) (Fig. 1B). In the presence of mitomycin C, a cell proliferation inhibitor, both VEGF-Grab and aflibercept showed successful inhibition of cell migration, and the rate of cell migration was lower in the VEGF-Grab group (regression coefficient, 12.9%/min in the VEGF-Grab vs. 17.8%/min in the aflibercept; P < 0.001, ANCOVA) (Fig. 1B). In addition, the tube formation assay revealed that both VEGF-Grab and aflibercept strongly suppressed the tube formation of...
**Figure 1.** Comparison of the in vitro anti-VEGF effects of VEGF-Grab and aflibercept. (A) Quantification of VEGF-A-induced cell proliferation/migration inhibition, defined as the closed area (%). (B) Quantification of VEGF-A-induced cell proliferation/migration inhibition with and without mitomycin C, defined as wound confluence (%), to measure wound closure. The rates of cell migration were calculated and compared between the drugs using ANCOVA. (C) Quantification of VEGF-A-induced tube formation inhibition, defined as the relative tube formation compared with VEGF-A only (%). MMC, mitomycin C; n.s., nonsignificant by Mann–Whitney U test. *P < 0.05, Mann–Whitney U test; **P < 0.01, Mann–Whitney U test; ***P < 0.001, ANCOVA.
FIGURE 2. In vivo antiangiogenic efficacy of both VEGF-Grab and aflibercept in the mouse OIR model. (A) Retinal flat-mount images of the neovascular tufts in the control (PBS solution only), VEGF-Grab, and aflibercept groups observed at 5 days following intravitreal injection. Scale bars: 1000 μm. (B) Magnified view of the area indicated by the red dashed rectangle in A. (C) Comparison of the neovascularization (NV) suppression among the control (PBS solution only), VEGF-Grab, and aflibercept groups. NV suppression (%) was defined as (neovascular area, μm²)/(total retinal area, μm²), divided by that of the contralateral eye (%). Data are presented as mean ± SEM. **P < 0.01, Mann–Whitney U test, control versus VEGF-Grab; ***P < 0.001, Mann–Whitney U test, control versus aflibercept; n.s., nonsignificant by Mann–Whitney U test, VEGF-Grab versus aflibercept. NS, neovascularization.

The in vivo antiangiogenic efficacy of VEGF-Grab was compared with that of aflibercept in OIR (Fig. 2) and laser-induced CNV (Fig. 3) models. Specifically, in the OIR model, NV suppression (determined by the decreasing rate of NV) was 42.78 ± 17.49%, 96.92 ± 10.53%, and 84.04 ± 3.79% in the control eyes injected with PBS, in the eyes treated with VEGF-Grab, and in those treated with aflibercept, respectively. Both VEGF-Grab and aflibercept showed superior suppression of retinal neovascularization as opposed to the control (control vs. VEGF-Grab, P < 0.01; control vs. aflibercept, P < 0.001; Mann–Whitney U test) (Fig. 2C). Also, VEGF-Grab showed regression of retinal neovascularization that was similar to that for aflibercept (P = 0.064, Mann–Whitney U test) (Fig. 2C). In the laser-induced CNV model, eyes treated with VEGF-Grab (CNV spot, n = 36) and aflibercept (CNV spot, n = 50) exhibited significantly smaller CNV sizes than eyes treated with the vehicle (CNV spot, n = 52) in the laser-induced CNV model (38473 ± 17251 μm² in control eyes; 19135 ± 9884 μm² in VEGF-Grab-treated eyes; 16617 ± 9767 μm² in aflibercept-treated eyes; control vs. VEGF-Grab P < 0.001; control vs. aflibercept P < 0.001; Mann–Whitney U test) (Fig. 3B). However, the size of CNV did not differ significantly between the VEGF-Grab and aflibercept groups (P = 0.302, Mann–Whitney U test) (Fig. 3B).

The in vivo retinal toxicity of VEGF-Grab and aflibercept was evaluated by light microscopy after dissection of the mouse eyeballs at 7 and 30 days following the injection of the vehicle, VEGF-Grab, or aflibercept (Fig. 4). Eyes treated with VEGF-Grab or aflibercept did not present significant retinal tissue abnormality when compared to both normal untreated eyes and vehicle-injected eyes. Furthermore, the retinal tissues of mouse eyeballs were also observed through TEM at 30 days after the intravitreal injection of vehicle, VEGF-Grab, or aflibercept (Fig. 4, rows 2–5). An increase in the number of focal vacuolization and Müller cells was seen in both the inner nuclear and inner plexiform layers of those eyes treated with VEGF-Grab or aflibercept similarly compared to normal untreated eyes and vehicle-injected eyes (Fig. 4, rows 4–5, column B). Otherwise, significant retinal tissue abnormality was not found in eyes treated with HUVECs (P < 0.05) (Fig. 1C), without a significant difference between the two compounds.
FIGURE 3. In vivo antiangiogenic efficacy of both VEGF-Grab and aflibercept in the rat laser-induced CNV model treated with PBS solution (control), VEGF-Grab, or aflibercept. (A) Representative image of CNV by retinal flat mount in the control, VEGF-Grab, and aflibercept groups observed at 14 days following injection. Scale bars: 100 μm. (B) Quantification of the CNV area. Data are presented as mean ± SEM. ***P < 0.001, Mann–Whitney U test, control versus aflibercept and control versus VEGF-Grab; n.s., nonsignificant by Mann–Whitney U test, comparing VEGF-Grab and aflibercept. N, total number of laser spots.

VEGF-Grab or aflibercept. Finally, neither adverse events nor signs of ocular inflammation were observed in the eyes treated with VEGF-Grab or aflibercept.

DISCUSSION

In the present study, VEGF-Grab showed efficacy comparable to that of aflibercept, both in vivo and in vitro, through the suppression of retinal and choroidal neovascularization, and it showed superior efficacy in in vitro proliferation/migration assay. Furthermore, similar to aflibercept, VEGF-Grab was not associated with a significant toxicity in the retina in the short- and long-term assays. Papadopoulos et al.25 compared the in vitro protein-binding affinities of VEGF-Trap, bevacizumab, and ranibizumab and reported that VEGF-Trap exhibited a markedly higher (about 100-fold) affinity to VEGF-A than the other drugs. In addition, they suggested that it could also bind to VEGF-B and PlGF, whereas the same could not be said for bevacizumab and ranibizumab. Stewart et al.13 predicted that the intravitreal VEGF binding affinity to VEGF-Trap would be maintained longer than that of ranibizumab (as the affinity of aflibercept at 79 days would be similar to that of ranibizumab at 30 days) in mathematical models. Given such results, VEGF-Trap and aflibercept (VEGF Trap-Eye) are thought to be anti-VEGF agents with powerful VEGF-binding affinity. As such, aflibercept is being actively used for the treatment of retinal diseases in clinical practice and has shown superior efficacy compared to ranibizumab and bevacizumab in clinical trials.5

Lee et al.17 developed VEGF-Grab by modifying the structure of VEGF-Trap to allow stronger binding to both VEGF and PlGF. They also showed that the downstream pathway of VEGF-Grab is the inhibition of VEGF-A-induced phosphorylation of VEGFR2 and ERK, similar to VEGF-Trap or aflibercept.17 Moreover, they conducted VEGF-induced migration and tube formation assays using HUVECs, and they found that VEGF-Grab3 (now called VEGF-Grab) could inhibit cell migration and tube formation at a greater rate than VEGF-Trap.17 In the current study, the potential efficacy of VEGF-Grab and that of the commercial product aflibercept were compared in an in vitro environment by reproducing both the cell migration assay and the tube formation assay. Indeed, VEGF-Grab was superior in the HUVEC
FIGURE 4. In vivo retinal toxicity of both intravitreal VEGF-Grab and aflibercept evaluated by light microscopy at 7 days following intravitreal injection into mouse eyeballs (1) and TEM at 30 days after intravitreal injection into mouse eyeballs (2–5). (A) Vehicle-treated eyes, (B) aflibercept-treated eyes, (C) VEGF-Grab-treated eyes. (1) Light microscopy images; (2) TEM, Bruch’s membrane and choriocapillaris; (3) photoreceptor cell layer; (4) inner nuclear and inner plexiform layers; (5) ganglion cell layer. BM, Bruch’s membrane; CC, choriocapillaris; INL, inner nuclear layer; IPL, inner plexiform layer; IS/OS, inner segment/outer segment of photoreceptor cell; GC, ganglion cell body; M, Müller cell; N, nucleus of photoreceptor cell; ONL, outer nuclear layer; OPL, outer plexiform layer; RPE, retinal pigment epithelium.

cell migration assay compared to aflibercept, whereas the two drugs were comparable in terms of the tube formation assay. These results, together with the higher binding affinity to VEGF/PIGF suggested in the previous study, imply that VEGF-Grab offers greater potential for VEGF suppression than aflibercept. Therefore, it may be the biologic agent with the greatest binding affinity to VEGF among all of the anti-VEGF agents currently used in the clinical practice.
The OIR mouse model is one of the preferred preclinical models for demonstrating the efficacy of new biological agents. In fact, it allows the modeling of increased vascular permeability and retinal neovascularization for diabetic retinopathy and retinopathy of prematurity (ROP).\textsuperscript{26–29} In our study, VEGF-Grab showed efficacy similar to that of aflibercept in the in vivo suppression of retinal neovascularization in the OIR model; therefore, it can be considered promising for the treatment of diabetic retinopathy and ROP. Mounting evidence from clinical trials suggests that aflibercept is one of the most powerful anti-VEGF agents for the treatment of diabetic macular edema\textsuperscript{2} and that it is not inferior to conventional laser therapy for proliferative diabetic retinopathy.\textsuperscript{30–32} In addition, it was shown to have a favorable outcome effect for ROP, although well-designed clinical trials have yet to be conducted.\textsuperscript{33,34}

Given its reproducibility and consistency, the laser-induced CNV mouse or rat model has been used in the preclinical testing of biological agents for the neovascular AMD pathology.\textsuperscript{35,36} In the present in vivo suppression study of choroidal neovascularization, VEGF-Grab showed efficacy comparable to that of aflibercept. Monthly (or bimonthly) doses of aflibercept have been shown to have similar efficacy and safety outcomes compared to monthly administration of ranibizumab in VIEW 1 and VIEW 2 studies,\textsuperscript{10} and VEGF-Grab may therefore offer good efficacy for the treatment of neovascular AMD that is not inferior to aflibercept.

Both light microscopy and TEM of rabbit eyes have been used for the preclinical safety screening of intravitreal agents.\textsuperscript{37–39} An in vivo retinal safety study of VEGF-Grab utilizing TEM found an absence of significant ultrastructural abnormality in the retina at 30 days after the intravitreal injection. This increases the likelihood of using this biologic molecule in human eyes without significant complications.

The results of present study suggest that VEGF-Grab represents a potential novel therapeutic biologic drug for VEGF-related ophthalmic diseases, including neovascular age-related macular degeneration and diabetic retinopathy. Given that the protein structure and molecular weight of VEGF-Grab are similar to those of aflibercept, it is reasonable to conclude that both the intravitreal pharmacokinetics and the intraocular safety profiles for both drugs would be similar in the human eyes.

Considering the results from the various clinical trials on diabetic retinopathy and neovascular age-related macular degeneration,\textsuperscript{1,5,7,10,30,40,41} aflibercept is among the most efficacious anti-VEGF agents used in the clinical practice. Indeed, aflibercept is known to have a higher affinity to both VEGF and PI GF than ranibizumab, but such affinity is supposed to be the reason for its superior efficacy and duration of action. Given that VEGF-Grab shows a higher affinity to both VEGF and PI GF than aflibercept, we believe that VEGF-Grab is as potent as, or even more efficacious than, aflibercept for the treatment of VEGF-related ophthalmic diseases. However, the actual clinical efficacy of VEGF-Grab should be demonstrated through further clinical trials.

A compound similar to aflibercept is conbercept, which was introduced in 2013. It is composed of the second Ig domain of VEGFR1 (D2) and the third and fourth Ig domains of VEGFR2 (D3 and D4) to the Fc region of human IgG1. A systematic review of six randomized controlled trials suggests that conbercept is more effective than ranibizumab in lowering the VEGF plasma level.\textsuperscript{32} In addition, conbercept was equivalent to ranibizumab in visual acuity with regard to a treat-and-extend protocol for the treatment of neovascular AMD.\textsuperscript{33} However, a comparison with aflibercept is still lacking. Theoretically, given that the structure of conbercept is similar to that of aflibercept, conbercept may have a high affinity to all VEGF isoforms and PI GF that is similar to that of aflibercept.\textsuperscript{34} Considering that VEGF-Grab showed superior binding affinity to VEGF-Trap, as well as antiangiogenic efficacy comparable to that of aflibercept, it is reasonable to expect that this novel agent would also show an efficacy either comparable or superior to that of conbercept, although further investigation through randomized clinical trials is necessary.

To conclude, compared to aflibercept, which is one of the most potent drugs for the treatment of age-related macular degeneration and diabetic retinopathy, VEGF-Grab, a novel anti-VEGF molecule, showed comparable in vivo efficacy in the suppression of retinal neovascularization and choroidal neovascularization without retinal toxicity, as well as superior in vitro efficacy. Thus, VEGF-Grab may represent a potential drug for the treatment of VEGF-related retinal diseases and could prove to be an alternative to aflibercept; however, future clinical trials to prove its clinical efficacy and safety are warranted.

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