Objective: To investigate the serum levels of vascular endothelial growth factor (VEGF) before and after intravitreal injection of conbercept or ranibizumab for neovascular age-related macular degeneration and polypoidal choroidal vasculopathy patients.

Methods: This study is a prospective, interventional case series and involved 28 patients, 18 treated with 0.5 mg of conbercept and 10 treated with 0.5 mg of ranibizumab. Serum concentrations of VEGF were determined by enzyme-linked immunosorbent assay before the injection and at 1 day, 1 week, and 1 month after anti-VEGF treatments.

Results: The baseline serum VEGF level of the ranibizumab group was 367.11 ± 311.87 pg/mL, whereas that of the conbercept group was 315.06 ± 170.88 pg/mL (P = 0.653). In the conbercept group, VEGF level significantly decreased to 36.32 ± 72.11 pg/mL at 1 day (P = 0.03) and returned to 136.55 ± 144.62 pg/mL at 1 week (P = 0.03). At 1 month, the concentration increased to 334.48 ± 197.41 pg/mL and showed no significant difference compared with the baseline. In the ranibizumab group, the serum VEGF levels were 292.42 ± 239.80 pg/mL, 282.60 ± 201.36 pg/mL, and 308.83 ± 266.89 pg/mL at 1 day, 1 week, and 1 month after intravitreal injection, respectively. There was no significant difference in the ranibizumab group at each detection time point (P = 0.45).

Conclusion: Conbercept significantly decreased serum VEGF level 1 day and 1 week after injection, but this effect was not sustained for 1 month. In contrast, ranibizumab had no significant effect on serum VEGF concentration changes. The reduction in serum VEGF by conbercept may affect its systemic safety profile.

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Neovascular age-related macular degeneration (AMD) is one of the leading causes of blindness in the aging population of developed countries.1,2 Polypoidal choroidal vasculopathy (PCV), a special subtype of AMD, is more common in Asian populations. Studies have demonstrated the crucial role of vascular endothelial growth factor (VEGF) in the process of neovascularization, which was thought to be a key pathological mechanism in exudative AMD characterized by growth of new blood vessels.3,4 Intravitreal injections of VEGF inhibitors have been thought to be the main way to block the neovascularization.5 In the past decade, several anti-VEGF medications have been introduced for the treatment of AMD, including bevacizumab (Avastin; Genentech, Inc, South San Francisco, CA), ranibizumab (Lucentis; Genentech, Inc and Novartis International AG, Basel, Switzerland), aflibercept (Eylea; Regeneron, Tarrytown, NY and VEGF-Trap Eye; Bayer AG, Leverkusen, Germany), and the newest drug introduced to ophthalmology called conbercept (KH902) (Lumitin; Chengdu Kanghong Biotech, Ltd, Sichuan, China).6–9

Ranibizumab, a recombinant, humanized monoclonal antibody Fab fragment, is the first VEGF inhibitor that was approved by the US Food and Drug Administration used for exudative AMD. The expanding application of
ranibizumab worldwide promotes the safety and systemic research. In recent years, several studies had reported the systemic VEGF level after intravitreal injection of ranibizumab in patients with AMD. In these studies, ranibizumab was proved to have a minimal effect on serum or plasma VEGF level 1 day after injection and recover shortly, although it may reduce the long-term systemic VEGF to abnormal levels on a pro re nata regimen.10–13 Comparably, the systemic VEGF level in serum or plasma of AMD patients treated within bevacizumab and aflibercept was detected and could last over at least 1 month.12–14

Conbercept is a soluble receptor decoy that combines the second Ig-like domain of VEGFR-1 and the third and fourth Ig-like domains of VEGFR-2 and is fused to the Fc portion of human IgG1.15 It has the highest binding affinity to VEGF-A165 and can bind all isoforms of VEGF-A, VEGF-B, and placentas growth factor (PIGF) with high affinity. The structural difference between conbercept and ranibizumab may lead to a different systemic influence. Although only having been used for 1 year, conbercept has been used so widely and become one of the first-line drugs for the treatment of AMD in China. Therefore, the systemic effects of intravitreal injection of conbercept require great attention.

In this study, we investigated systemic VEGF level changes before and after intravitreal injection of conbercept and ranibizumab for exudative AMD and PCV patients. To the best of our knowledge, it is the first report on the influence of intravitreal injection of conbercept on systemic VEGF level.

Methods

Subjects

The present study recruited 28 patients with AMD (17 with neovascular AMD and 11 with PCV) who received intravitreal injection of ranibizumab or conbercept. The study protocol was approved by the Ethical Committee of Peking University People’s Hospital and was conducted in accordance with the tenets of the Declaration of Helsinki. Informed consent was obtained from each study subject. All of the participants provided their written informed consent to participate in this study. Exudative AMD and PCV were exactly diagnosed with fluorescein or indocyanine angiography by experienced retina specialists. Only patients who had not received any treatment for AMD for at least 3 months were enrolled. All the participants enrolled in the present study were treatment naive. Patients with any other ocular vasculopathy or systemic disorders with pathological features were excluded from the present study.

Treatment

Patients with AMD who had no history of intravitreal injection in the past 3 months were given an intravitreal injection of ranibizumab or conbercept. The procedure of intravitreal injection was performed by the same retina specialist (Dr. H. Qi). Of the 28 enrolled participants, 18 eyes of 18 patients received treatment with conbercept (the conbercept group, 0.5 mg/0.05 mL) and 10 eyes of 10 AMD patients were treated with ranibizumab (the ranibizumab group, 0.5 mg/0.05 mL). All intravitreal injections were performed under sterile conditions in the operating room after topical administration using povidone–iodine. Topical antibiotic levofloxacin was given before or after injection and was continued until 3 days after injection.

Sample Collection and Preparation

Blood samples were obtained within 3 days of intravitreal injection, and then 1 day, 1 week, and 4 weeks after intravitreal injection. Blood samples were drawn by venous puncture from all participants. For serum preparation, all blood samples were centrifuged at 3,000 rpm for 10 minutes within 1 hour of collection. The serum samples were stored at −80°C until the assay, which was performed within 1 month of sampling.

Measurement of VEGF Concentration

Serum VEGF concentrations were determined by enzyme-linked immunosorbent assay of human VEGF (DVE00, Quantikine; R&D Systems, Minneapolis, MN) according to the instructions given by the manufacturer. The Quantikine Human VEGF Immunoassay is a 4.5-hour solid-phase enzyme-linked immunosorbent assay designed to measure VEGF_165 levels in serum, plasma, and cell culture supernates. Results of the standard curves obtained for human VEGF and recombinant
human VEGF$_{121}$ indicate that this kit can be used to determine relative mass values for natural human VEGF. The lower limit of VEGF detection was 9.0 pg/mL. All VEGF assays were measured by the same skillful technician, and all samples were analyzed together with duplication.

Statistical Analysis

All data on VEGF concentration are presented as mean ± standard deviation. Data were compared using paired sample $t$-test for different time points in the same group. Independent sample $t$-test was performed between two groups. All statistical analyses were performed using SPSS (Version 19.0; International Business Machines Corporation, Armonk, NY). All tests were 2 tailed, and statistical significance was set at a $P$ value of <0.05. As mentioned before by Wang et al, the serum VEGF concentrations of less than the lower limit of detection were considered to be 0 for analytic purpose. A sample size power analysis was performed by Power Analysis (PASS, NCSS, Kaysville, UT). The significance level (alpha) was set to 0.05 using a 2-sided, 2-sample $t$-test.

Results

The baseline characteristics of the participants are described in Table 1. No age or gender differences were found among the 2 treatment groups. All the patients enrolled were observed for 1 month, and no ocular or systemic adverse events were observed. The baseline concentration of VEGF in the serum of AMD patients before the injection of ranibizumab was 367.11 ± 311.87 pg/mL (range, 71.12–879.51 pg/mL; n = 10), whereas the concentration in the conbercept group was 315.06 ± 239.80 pg/mL (range, 80.03–601.92 pg/mL; n = 18). There were no significant differences in baseline concentrations or characteristics between the patients of the 2 groups (Figure 1, $P = 0.653$). The mean (±standard deviation) serum level of VEGF for the conbercept group significantly decreased to 36.32 ± 72.11 pg/mL (range, 0–248.59 pg/mL; $P < 0.001$; n = 18) 1 day after the injection. For 12 of 18 patients included in this group, the concentration of serum VEGF even reduced to the values under the minimum detectable dose. Even 1 week after the injection, the serum VEGF level was still significantly lower than baseline with a mean value of 136.55 ± 144.62 pg/mL (range, 0–398.18 pg/mL; $P = 0.03$; n = 10). Concentrations under the lower detectable limit were observed in 2 of the 18 enrolled patients. The mean serum VEGF concentration increased to 334.48 ± 197.41 pg/mL (range, 45.71–706.96 pg/mL; n = 17) 1 month after the injection, which was close to the baseline level and showed no significant difference with the mean value before injection (Figure 2; $P = 0.243$).

In the ranibizumab group, no significant effect on the VEGF concentration of serum could be observed ($P = 0.45$). The mean serum VEGF level was 292.42 ± 239.80 pg/mL (range, 68.87–750.34 pg/mL; n = 10) 1 day after the follow-up, which seemed to be a bit lower than the baseline value but not significantly different. During the follow-up, the serum VEGF levels were 282.60 ± 201.36 pg/mL (range, 80.03–601.92 pg/mL; n = 8) after 1 week and 308.83 ± 266.89 pg/mL (range, 47.30–811.31 pg/mL; n = 9) after 1 month (Figure 3). The reduction in serum VEGF concentration after intravitreal injection of ranibizumab did not occur in the present study.

For the comparison between the 2 groups, the VEGF concentration was no different before the injection and 1 month after the injection. However, the serum VEGF levels after 1 day and 1 week in the conbercept group were significantly lower than those in the ranibizumab group (after 1 day, $P = 0.03$; after 1 week, $P = 0.03$) (Table 2). A sample size power analysis was performed. Sample sizes of 18 in Group 1 and 10 in Group 2 achieved 86% power to detect a difference in mean changes of 240.0, and a correlation between measurement pairs of 0.100.

Discussion

The serum or plasma levels of VEGF have been estimated in various ocular diseases, especially in AMD. To the best of our knowledge, the current study is the first evaluation of the serum concentration of VEGF in patients treated with conbercept. Our study showed a significant reduction in serum VEGF concentration in AMD patients after intravitreal injection of conbercept, which lasted for 1 week but regressed at 1 month. This study provided a further understanding of the systemic effect of conbercept and

<table>
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<th>Characteristics</th>
<th>Conbercept Group</th>
<th>Ranibizumab Group</th>
<th>$P$</th>
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<td>Patients (n)</td>
<td>18</td>
<td>10</td>
<td>—</td>
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<td>65.56 ± 10.73</td>
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Mean ± standard deviation were evaluated as the baseline data for both the groups; $P < 0.05$ was considered as statistically significant.
offered information for comparison of conbercept and ranibizumab from the point of view of drug therapy. As we all know, although the anti-VEGF agents were first developed in oncology treatment, they have markedly revolutionized the treatment of retinal disease, especially for AMD. The worldwide application of VEGF inhibitors offered invaluable help for preventing the visual acuity defect in exudative AMD or PCV patients. To better understand the pharmacological action and potential systemic effectiveness of the VEGF

![Fig. 1. Serum concentration of VEGF at baseline in neovascular AMD or PCV patients treated with ranibizumab or conbercept. The serum VEGF levels were not significantly different between the 2 groups (P = 0.653).](image1)

![Fig. 2. Serum concentration of VEGF before and after intravitreal injection of conbercept in patients with neovascular AMD or PCV. One day after injection, serum VEGF level decreased and significantly lower than that of baseline (**P = 0.03), and the VEGF concentration began to increase at 1 week, but it remain significantly lower than that of baseline (**P = 0.03). One month after intravitreal injection of conbercept, the serum VEGF level returned to the baseline level (**P = 0.243).](image2)
inhibitors, several studies had focused on the serum or plasma VEGF concentrations in patients with various diseases.\textsuperscript{10–14,17,18} Conbercept was the latest anti-VEGF medicine introduced to ophthalmologists, and it was designed and produced in China as an alternative choice for AMD therapy. It was approved by the China Food and Drug Administration for intraocular use in the treatment of AMD in December 2013, and it has been used in clinical practice for more than 1.5 years.\textsuperscript{19} It was designed to be a prolonged inhibitor of all VEGF isoforms with the highest affinity, which is similar as that of aflibercept and much higher than ranibizumab and bevacizumab.\textsuperscript{20,21} Additionally, the safety and efficacy of injections of conbercept have also been confirmed by a randomized, double-masked, multicenter study.\textsuperscript{7} Given it determined the safety and efficacy profile, and lower price, conbercept has gained attention as a promising alternative treatment for AMD.\textsuperscript{19} However, no systemic VEGF concentrations for intraocular use of conbercept have been reported to date.

The pharmacokinetic characteristics are of significant importance for local effects and systemic off-target effect estimation. For anti-VEGF agents, the IC\textsubscript{50} (half inhibitory concentration) determines the systemic concentration–time profile, which is an indicator of systemic accumulation of a certain drug. The IC\textsubscript{50} value of conbercept is similar to that of aflibercept, which is 38-fold to 48-fold greater than that of ranibizumab and bevacizumab.\textsuperscript{21,22} It implicated that the increased systemic exposure probably happens in patients treated with conbercept. The prolonged suppression of systemic VEGF levels resulting from intravitreal injection of VEGF inhibitors might raise the potential possibility.

Table 2. Serum VEGF Concentrations Obtained From Neovascular AMD or PCV Patients During the Follow-up Period Before and After Intravitreal Injection

| Group      | Serum VEGF Concentration (pg/mL) | 1 Day     | 1 Week    | 1 Month   |  | P  |
|------------|----------------------------------|----------|-----------|-----------|  |    |
| Baseline   | 315.06 ± 170.88                  | 36.32 ± 72.11 | 136.55 ± 144.62 | 334.48 ± 197.41 | 0.017 |
| Conbercept | (n = 18)                         | (n = 18) | (n = 10)  | (n = 17)  |   |    |
| Ranibizumab| 367.11 ± 311.87                  | 292.42 ± 239.80 | 282.60 ± 201.36 | 308.83 ± 266.89 | 0.45 |
| (n = 10)   |                                  | (n = 10) | (n = 8)   | (n = 9)   |   |    |
| P          | 0.653                            | —        | —         | —         |   |    |

Mean ± standard deviation were evaluated as the baseline data for both groups; \( P < 0.05 \) was considered as statistically significant.
of unwanted systemic effects. Moreover, considering most patients with AMD are elderly, the evaluation of serum VEGF concentrations after intravitreal injection of conbercept or ranibizumab is particularly important.

In this study, a significant reduction in serum VEGF 1 day and 1 week after an injection of conbercept was found, which seemed to recover to baseline at the 1-month follow-up. The off-target effect in the first week after intravitreal treatment was in line with the reported variation from aflibercept, but it was not sustained 1 month after injection. The serum levels of VEGF in the conbercept group showed different change within 1 month after therapy from that of ranibizumab or aflibercept reported before, which may be influenced by the pharmacodynamic features of these agents. Although there was no in vivo study, the studies in rabbits have shown similar results. The intravitreal half-life of conbercept was 4.2 days in an experiment with rabbits, which was shorter than the vitreous half-life of aflibercept that was as high as 4.79 days in rabbits. Another study revealed the shortest intraocular half-life of ranibizumab as 2.88 days, which was much shorter than that of conbercept and aflibercept. Fc fragment played an important role in penetrating from the vitreous to the retina and choroid, especially for larger molecule anti-VEGF agents, such as aflibercept and bevacizumab.

At the systemic level, the FcRn–Fc interaction was thought to extend the half-lives of molecules with an Fc fragment in circulation. This result may better explain the longer persistence of conbercept in serum, as well as aflibercept and bevacizumab, which decreased the serum VEGF concentration directly. For ranibizumab with no Fc fragment, the serum half-life was much shorter and persisted only for a few hours. Moreover, the small molecular structure made ranibizumab easier to filter by the kidney. Longer serum half-life indicates longer continuous effects on the serum VEGF level, which would have subsequent systemic effects.

Compared with aflibercept, conbercept has an additional Domain 4 of VEGF Receptor-2, which plays a certain role in decreasing the positive charge of the molecule and may lead to a decrease in adhesion to the extracellular matrix. It helped to explain the high serum-free VEGF affinity of conbercept indirectly. However, until now, no head-to-head study of aflibercept versus conbercept or ranibizumab versus conbercept had been performed.

In contrast to the conbercept, no significant suppression of serum VEGF level was found in the ranibizumab group during the 1-month observation. The stable systemic VEGF concentration in the ranibizumab group was consistent with previous studies. In the recent studies by Yoshida et al and Zehetner et al, no significant reductions in after-injection plasma VEGF concentration were observed in the ranibizumab group. Both serum and plasma VEGF levels detected showed no significant change at 1 week and 1 month after intravitreal injection of ranibizumab in the study performed by Wang et al, and no significant change in VEGF levels was described in their study. The shorter half-life and much smaller IC50 explained the quick metabolism and less systemic accumulation of ranibizumab, which may explain the quick regression of serum VEGF levels.

Although we did not observe any systemic side effects in our study, the decreased systemic VEGF level might increase potential systemic risks and could not be ignored. Vascular endothelial growth factor is a multifunctional cytokine with many other actions on the endothelium that regulate biological function in healthy adult vessels and the systemic adverse events (SAEs) of the VEGF inhibitors, including hypertension, proteinuria, and thromboembolic incidents. A multitude of anti-VEGF agent studies have concerned the SAEs attributed to the systemic exposure to VEGF inhibitors. In terms of serum or plasma VEGF levels, recent studies have considered the systemic effect of ranibizumab to be the lowest. An imbalance in SAEs was reported in the comparative trial of bevacizumab and ranibizumab, which may indicate the potential biological connection between SAEs and systemic VEGF levels. In the present study, patients with AMD treated with conbercept or ranibizumab were evaluated for their serum VEGF levels. Because the correlation analysis between SAEs and serum VEGF levels require a larger sample and detailed SAEs data for a long follow-up period, the current study only showed serum VEGF concentrations at the given time points. In Phase 2 study of the AURORA study, which concerned conbercept, no systematic SAEs was judged to be related to the study drug or to the study procedure. However, the potential inner relationship between SAEs and systemic exposure to abnormally lower serum VEGF should not be ignored, and a larger scale prospective, randomized, clinical trial is needed to better understand the relationship between systemic VEGF levels and SAEs for various anti-VEGF agents, although it may not be easy to achieve.

There were limitations of the current study. The present study used a prospective design, but the enrolled patients in the two groups were not randomized but administered according to the patient’s wish. The sample size was also small, and a sample size power analysis had been performed. Although the enrolled standard allowed for the patients who received no treatment in the past 3 months, all the participants enrolled were naive to treatment by chance, which helped to make the drug influence much clearer. Because the AMD patients were mostly elderly people, a deeper investigation of the systemic effects of anti-VEGF agents was required.
In conclusion, the serum concentration of VEGF decreased 1 day and 1 week after intravitreal injection of conbercept, but the reduction in systemic VEGF was not sustained for 1 month. In contrast, patients treated with ranibizumab did not experience a reduction in VEGF and had no significant decrease in systemic VEGF concentration at each time point through the 1-month follow-up. The reduction of VEGF in serum requires enough attention for its potential effect on the systemic safety of anti-VEGF agents.

**Key words:** vascular endothelial growth factor, conbercept, ranibizumab, age-related macular degeneration, polypoidal choroidal vasculopathy, systemic effects.

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