TRIPLE THERAPY FOR CHOROIDAL NEOVASCULARIZATION DUE TO AGE-RELATED MACULAR DEGENERATION
Verteporfin PDT, Bevacizumab, and Dexamethasone

ALBERT J. AUGUSTIN, MD, STEPHAN PULS, MD, INDRE OFFERMANN, MD

Purpose: To evaluate the efficacy and safety of triple therapy with verteporfin photodynamic therapy (PDT), dexamethasone, and bevacizumab in choroidal neovascularization (CNV) secondary to age-related macular degeneration (AMD).

Methods: This prospective, noncomparative, interventional case series included 104 patients. Verteporfin PDT was administered with a reduced light dose (42 J/cm², accomplished by light delivery time of 70 seconds). Approximately 16 hours after PDT, dexamethasone (800 μg) and bevacizumab (1.5 mg) were injected intravitreally. Patients attended follow-up visits every 6 weeks, undergoing visual acuity and intraocular pressure measurement, slit-lamp and ophthalmoscopic examination, and optical coherence tomography (OCT). Fluorescein angiography was performed every 3 months or earlier if OCT showed significant edema.

Results: All 104 patients received one triple therapy cycle (5 patients received a second triple treatment due to remaining CNV activity). The triple therapy was complemented in 18 patients (17.3%) by an additional intravitreal injection of bevacizumab. The mean follow-up period was 40 weeks (range, 22–60 weeks). Mean increase in visual acuity was 1.8 lines \((P<0.01)\). Mean decrease in retinal thickness was 182 μm \((P<0.01)\). No serious adverse events have been observed.

Conclusion: In most patients with CNV due to AMD, triple therapy results in significant and sustained visual acuity improvement after only one cycle of treatment. In addition, the therapy offers a good safety profile, potentially lower cost compared with therapies that must be administered more frequently, and convenience for patients.

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As new therapies have become available to treat patients with choroidal neovascularization (CNV) due to age-related macular degeneration (AMD), it is clear that no single therapy addresses the multifactorial pathogenesis of the disease. CNV progresses from age-related changes in the retina and supporting tissues. With age, the cells of the retinal pigment epithelium (RPE) are less able to detoxify, and cellular debris (drusen) accumulates between Bruch membrane and the RPE.1,2 As Bruch membrane and the adjacent choriocapillaris age, they become brittle, reducing the size of the choroidal capillaries, and in turn affect metabolic activity and lead to hypoxia.1–3 This choroidal vascular atrophy with the associated inflammatory response is thought to be the principal cause of dry AMD development.2,3 Immunologic signals associated with acute vascular compromise may lead to
development of CNV.\textsuperscript{2–4} Recent studies support the concept that genetics (especially genetic variation in the complement regulatory gene factor H [HFI/CFH]) predispose individuals to AMD. This polymorphism is in a region of complement factor H that binds heparin and C-reactive protein. CFH has been identified as a key regulator of the complement system of innate immunity. This adds to the hypothesis that AMD results from an aberrant inflammatory process that includes inappropriate complement activation.\textsuperscript{5,6}

Links between CNV and chronic infection with cytomegalovirus and \textit{Chlamydia pneumoniae} have also been found in patients with AMD.\textsuperscript{7,8} In CNV, tissue ischemia and/or inflammation from either age-related changes or chronic infection triggers angiogenic signal molecules such as vascular endothelial growth factor (VEGF). Vascular endothelial cells receive these signals, which initiate a proteolytic cascade\textsuperscript{9} that results in neovascularization. Although new vessel growth and maturation are complex processes involving multiple stimuli, VEGF appears to be a particularly important signal\textsuperscript{10–13} and was found to be overexpressed in RPE cells of eyes with AMD at autopsy and in RPE cells from CNV membranes obtained at surgery.\textsuperscript{11,12}

An ideal therapy would eradicate existing CNV, as well as reduce inflammation and VEGF expression to prevent further CNV growth, with one treatment. No single therapy possesses all of these modes of action and the safety and convenience of one treatment cycles, so a combination strategy is warranted. Combination therapy strategies are common in oncology, from which anti-VEGF therapy originated. Anti-VEGF therapy has proved beneficial in treating some types of cancer, but only when combined with chemotherapy.\textsuperscript{14}

Studies of intravitreal anti-VEGF monotherapies (pegaptanib, Macugen, OSI Pharmaceuticals, NY; and ranibizumab, Lucentis, Genentech, San Francisco, CA) in the treatment of CNV, while showing beneficial effects in visual acuity (VA), with remarkable improvement for ranibizumab when given monthly, reveal that therapy must be frequently administered for a prolonged but unknown period of time to maintain the VA benefit.\textsuperscript{15} Patients with all lesion compositions treated with ranibizumab monthly for 3 months, and then quarterly to month 12, showed no improvement from baseline at month 12, in contrast to studies of monthly ranibizumab, which showed vision improvement at 12 months. Thus, anti-VEGF appears to inhibit continued neovascularization but not destroy existing CNV. Prolonged, frequent anti-VEGF therapy, with the associated additional safety risk, cost, and lack of convenience, is untenable. An additional concern is the potential for upregulation of VEGF receptors or compensatory overproduction of VEGF itself with prolonged use of anti-VEGF therapy, which could result in a rebound effect at the discontinuation of treatment. Such homeostatic mechanisms are well-known with other therapies.

Monotherapy studies of photodynamic therapy (PDT) with verteporfin (Visudyne; Novartis AG) have shown VA benefit with a limited number of treatments.\textsuperscript{16–18} However, on average this benefit is a reduced risk of VA loss, rather than VA improvement, and as such is unsatisfactory.

Anti-VEGF and PDT with verteporfin possess different mechanisms of action that may work synergistically, with their combination potentially improving VA with a limited and finite number of treatments. In addition, verteporfin therapy has been combined in practice with steroids such as intravitreal triamcinolone and dexamethasone, which have a third anti-inflammatory complementary mechanism of action. Open-label studies of verteporfin-steroid combinations show VA improvement with few treatments.\textsuperscript{19–26}

Triple therapy with verteporfin PDT, anti-VEGF, and steroid components has a good rationale based on their different mechanisms of action, as well as promising preliminary combination study results.\textsuperscript{27} Verteporfin PDT, with its unique angio-occlusive effect, is essential in combination therapy with anti-VEGF and/or steroid drugs. Verteporfin PDT, which selectively damages the cytoskeleton of the endothelial cells of neovessels, leading to their angio-occlusion, eradicates existing CNV, eliminating the source of VA deterioration. Reactions to this CNV eradication, however, include initiation of an inflammatory response and upregulation of VEGF and other growth factors.\textsuperscript{28} These reactions may prevent hypoxia-induced retinal damage, promote surrounding choroidal vessel recovery, and encourage maturation of CNV that is less permeable and less susceptible to reactivate neovascularization, but they may also lead to recurrent CNV growth, which limits VA benefit and necessitates additional treatment.

The addition of anti-VEGF therapy blocks the effects of VEGF that could be overexpressed by the pathogenesis of CNV and by the effect of verteporfin PDT. Anti-VEGF and verteporfin PDT combinations have the potential of destroying both the new and existing CNV.

The addition of steroid, the only one of the three therapies with anti-inflammatory properties, limits any further VEGF upregulation initiated by inflammation. While intravitreal triamcinolone has been combined with verteporfin PDT in several studies and benefit in VA was observed, steroid-induced intraocular pressure (IOP) increases were also observed.\textsuperscript{20,23,25,26}
Triamcinolone that is injected as a suspension has prolonged effects, particularly increased IOP. Dexamethasone, which is injected as a solution, is more rapidly cleared from the vitreous, as there is no sustained release due to suspension, and has potential for reduced steroid side effects. Beyond the anti-inflammatory effects, dexamethasone has other pharmacologic effects that may contribute to long-lasting, sight-saving treatment of CNV. Dexamethasone has been shown to have antifibrotic, antiproliferative, and antimigration properties. Furthermore, its antiproliferative effects are reduced in the presence of VEGF, so combination of dexamethasone with anti-VEGF therapy may assist with dexamethasone’s antiproliferative effects. Dexamethasone may also reduce endothelial dysfunction and inhibit VEGF-induced vascular leakage. On the molecular level, dexamethasone exerts its anti-inflammatory effect by interfering with the activation of proinflammatory genes without affecting factors that inhibit inflammation. We hypothesize that all these differences versus triamcinolone may lead to a better benefit/risk profile for dexamethasone.

The present case series investigates the preliminary efficacy and safety of triple therapy with verteporfin PDT, intravitreal bevacizumab (Avastin, Genentech, San Francisco, CA), and intravitreal dexamethasone in patients with wet AMD. All lesion types and all lesion sizes regardless of fibrotic composition were included. Verteporfin PDT was administered with a reduced light dose (standard fluence rate of 600 mW/cm² administered for 70 seconds instead of 83 seconds, corresponding to approximately 84% of the standard light dose) to limit potential choroidal damage. Bevacizumab was chosen as the anti-VEGF therapy. It is a full-length antibody that binds all isoforms of VEGF and should, in theory, produce results similar to those reported for ranibizumab, which was not approved by any regulatory agency for use in humans at the time of the present study. Bevacizumab and dexamethasone components of the triple therapy procedure and potential risks. The study protocol adhered to the European Good Clinical Practice Guidelines and the Declaration of Helsinki.

PDT with verteporfin (Visudyne, Novartis, Basel, Switzerland) was performed according to the recommended standard procedure, except that the time of light delivery was reduced to 70 seconds from 83 seconds, thereby delivering a light dose of 42 J/cm² instead of 50 J/cm². Within a mean of 16 hours after PDT (±1.5 hours), patients received retrobulbar anesthesia, and the ocular surface was disinfected using povidone iodine solution. Retrobulbar anesthesia was used to exclude eye movements and to avoid lens or retinal damage due to fixation problems of the eye. A monoport vitrectomy (0.5 mL) was performed using a 25-gauge vitrector to avoid reflux through the incision after dexamethasone and bevacizumab were injected. Eight hundred micrograms of dexamethasone (taken from Fortecortin Inject 4 mg, Merck, Germany) in a volume of 0.2-mL solution was administered intravitreally through the 25-gauge incision using a 27-gauge needle.

Bevacizumab (Avastin, Genentech) was given intravitreally as 1.5 mg in a volume of 0.06 mL through the 25-gauge incision using a 27-gauge needle. The dose of bevacizumab was slightly higher than that reported in studies of monotherapy intravitreal bevacizumab in patients with AMD because we used vials that had been frozen. Frozen storage of bevacizumab has been shown to have antifibrotic, antiproliferative, and antimigration properties. Furthermore, its antiproliferative effects are reduced in the presence of VEGF, so combination of dexamethasone with anti-VEGF therapy may assist with dexamethasone’s antiproliferative effects. Dexamethasone may also reduce endothelial dysfunction and inhibit VEGF-induced vascular leakage. On the molecular level, dexamethasone exerts its anti-inflammatory effect by interfering with the activation of proinflammatory genes without affecting factors that inhibit inflammation. We hypothesize that all these differences versus triamcinolone may lead to a better benefit/risk profile for dexamethasone.

**Methods**

Patients with all types of choroidal neovascularization secondary to AMD were included in this prospective interventional case series. At baseline, all patients underwent a standardized ophthalmologic examination, including best-refracted VA measurement using Snellen charts, slit-lamp, and fundus examination, and assessment of IOP. Optical coherence tomography (OCT) was performed to assess retinal thickness. Baseline 1-mm central retinal thickness was measured by OCT (Stratus OCT, Carl Zeiss Meditec) using 6-diagonal fast and slow 6-mm scans. IOP was determined weekly until 4 weeks, then in 6-week intervals. Fluorescein angiography (FA) was performed to identify lesion type and location and to determine whether active CNV leakage was present. Laser interferometry was used successfully to determine retinal function before cataract surgery due to the ability to circumvent lens opacities. All patients were re-evaluated every 6 weeks.

After oral informed consent was given, each participant signed a written consent form, which described the experimental nature of the bevacizumab and dexamethasone components of the triple therapy procedure and potential risks. The study protocol adhered to the European Good Clinical Practice Guidelines and the Declaration of Helsinki.
zumab was shown to reduce anti-VEGF activity. The dexamethasone and bevacizumab injections were performed using an operating microscope; any direct light exposure of the treated eye was avoided. All injections were administered in the operating room. A paracentesis was not required for any of the procedures.

Patients were scheduled for follow-up visits at 6-week intervals and underwent identical examination procedures, including best-refracted VA measurement, IOP documentation, slit-lamp and ophthalmoscopic examination, and OCT. FA was performed every 3 months or earlier if OCT showed significant edema.

The primary efficacy variable was defined as change in VA from baseline to the last visit as measured using Snellen VA charts. The mean VA at the final follow-up visit was compared with baseline measures using the paired t-test to test for statistical significance.

Results

A total of 104 patients diagnosed with CNV due to AMD were treated with triple therapy and included in the analyses. The mean age of patients was 76.5 years; 60.6% were women. Baseline lesion types were evenly distributed: predominantly classic (22.1%), minimally classic (38.5%), and occult (39.4%). Mean lesion greatest linear dimension was 2650 μm.

All 104 patients received an initial triple therapy cycle. Eighteen of those patients received an additional intravitreal injection of bevacizumab (mean of 15 weeks [range 12 to 32 weeks] after triple therapy) for retina modeling—we have coined the term retina modeling to describe the reduction of residual or recurrent edema on OCT without angiographically detectable CNV activity. In contrast, in five cases, we employed a second cycle of triple therapy because we sought to extinguish recurrent, angiographically detectable CNV activity.

The mean follow-up period was 40 weeks (range, 22–60 weeks).

Baseline mean VA was 20/126 (0.802 logMAR) (8.7% of the patients had a VA of 20/40 or better at baseline). Visual acuity improved in most patients, with a mean increase of 1.8 lines (P < 0.01) (Figure 1). The mean follow-up VA score was 20/85 (0.625 logMAR) (28.8% of the patients had a VA of 20/40 or better at last follow up). A total of 39.4% of the patients gained 3 or more lines. A total of 3.8% lost 3 or more lines.

A mean decrease in retinal thickness of 182 μm (P < 0.01) was observed (Figure 2). Mean retinal thickness was 463.5 μm at baseline and 281 μm at follow-up.

The triple therapy treatment including verteporfin PDT and intravitreal steroid (dexamethasone) and anti-VEGF (bevacizumab) injections was well tolerated. No patient had a severe or serious adverse event, and no ocular events were observed. No increases in IOP were observed. It has to be emphasized that intravitreal injections bare the risk of endophthalmitis.

Figure 3 shows FA and OCT images of a minimally classic lesion before and 24 weeks after one triple therapy cycle and no further treatment. The patient’s VA improved from 20/200 to 20/160, no CNV activity was evident, and edema completely resolved (central retinal thickness decreased from 530 μm to 300 μm).

Figure 4 shows baseline and follow-up FA and OCT images of another minimally classic lesion. Triple therapy led to CNV closure and VA improved from 20/100 to 20/50. Due to some remaining edema, a second bevacizumab injection was administered at...
18 weeks follow-up. This therapy led to complete edema resolution (overall central retinal thickness decreased from 427 μm to 193 μm) and a further improvement in VA to 20/32 at 24 weeks follow-up.

**Discussion**

After one cycle of triple therapy (verteporfin PDT + bevacizumab + dexamethasone) mean VA significantly improved by 1.8 lines in 104 patients with CNV due to AMD. Up to now the mean follow-up is 40 weeks. In 18 patients the triple therapy was complemented by an additional intravitreal injection of bevacizumab, what we call retina modeling. Five patients were retreated with triple therapy due to recurrence of CNV activity. Retinal thickness significantly decreased. These results suggest that the triple therapy regimen used in this case series leads to VA benefit, as well as anatomic improvement, after only one treatment cycle of triple therapy. The safety profile of this triple therapy was good; no unexpected safety issues and no IOP increases were observed.

VA improvement in this case series was similar to that reported for patients with AMD treated with monthly intravitreal bevacizumab after 3 months follow-up, suggesting that one triple therapy cycle may lead to functional benefit similar to monthly monotherapy treatments with an anti-VEGF antibody. The change in retinal thickness was also similar between the studies. These studies are not directly comparable because one monthly intravitreal bevacizumab study included more patients (266) than our case series (104 patients) and the follow-up period was shorter (3 months) than ours (10 months). The other intravitreal bevacizumab study included a similar number of patients (50 patients/53 eyes), but again the follow-up period was shorter (3 months). Nevertheless, the results suggest that similar benefit may be gained from one triple therapy cycle compared with up to three anti-VEGF monotherapy courses, with sustained benefit from the triple therapy, in contrast to the likely need for continued additional anti-VEGF monotherapy cycles to maintain the observed benefit.
We postulate that the sustained VA improvement observed in the present case series is due to termination of CNV by verteporfin PDT combined with inhibition of inflammation by dexamethasone and blockage of continued neovascularization due to VEGF by bevacizumab. The role of verteporfin PDT could be likened to that of surgical debulking of a tumor before chemotherapy. The combination of these three therapies with their complementary mechanisms of action, addressing multiple aspects of CNV pathogenesis, may be the key to effective, satisfactory, long-term benefit for patients with CNV due to AMD.

Monotherapy anti-VEGF may not result in sustained benefit without continuing treatment courses because the underlying condition, the existing CNV, is not eradicated. Monotherapy verteporfin PDT may result in unsatisfactory benefit because it does not address the edema and inflammation aspects of CNV, and its side effects might enhance them. The addition of a steroid anti-inflammatory agent to verteporfin PDT, which has been studied in small open-label trials, showed promising results, but the anti-inflammatory agent most used in these studies, triamcinolone, was also found to increase IOP and other associated safety concerns. In the present case series, dexamethasone was used both to ameliorate these safety concerns and to take advantage of its additional effects, particularly its antifibrotic and anti-VEGF effects. It appears that dexamethasone does contribute to a better safety profile, as none of the adverse effects observed with triamcinolone was seen with this triple therapy. We administered the dexamethasone 16 hours after PDT because PDT’s efficacy depends upon immunologically mediated processes which are established within a 16-hour period. Delaying 16 hours before administering dexamethasone ensures PDT’s therapeutic effects but prevents PDT-mediated overactivation of the inflammatory cascade.

We also postulated that a reduced light dose of PDT would result in a better safety profile, due to limiting potential choroidal damage. Overall, the triple therapy treatment was well tolerated, lending credibility to our hypotheses.

The present case series is limited by the relatively few patients and lack of a control arm. Another consideration is the vitrectomy performed to administer the bevacizumab and dexamethasone components of the triple therapy. Vitrectomy was done to ensure that the drug doses into the eye were appropriate for effect and to avoid early and late reflux of the vitreous, as well as the drug components. It is possible that the vitrectomy procedure itself may reduce inflammatory mediators and VEGF, although vitrectomy has not been demonstrated to have these therapeutic effects.

In the long term, continued monthly anti-VEGF monotherapy, which is expected to be required to maintain VA benefit, entails continued safety risks associated with repeated intravitreal injections, cost, and inconvenience to patients. The latter could lead to lack of compliance, precluding patients from completely benefitting from anti-VEGF monotherapy. Combined therapy, such as the triple therapy used in this case series, may be a better option in terms of durability, safety, and cost to patients and to the medical system as a whole.

The treatment regimen used in this case series may
not be optimal. Dosages and time intervals may need to be refined. Further, we cannot assess the individual contribution of each of the components of this regimen. We opted to use 800 μg of dexamethasone because a complete blockade of the inflammatory cascade is desired. We should note that the safety of this 800 μg dose has not been demonstrated until now. Furthermore, while we utilized somewhat invasive methods retrobulbar anesthesia, preinjection limited vitrectomy and performed them in an operating room setting simpler methods may prove more practical. More concentrated dexamethasone preparations would facilitate simple intravitreal injections in the office using a 30-gauge needle. Though we cannot prove that the vitrectomy did not contribute to the findings in these patients, we believe that the therapeutic effects contributed by the vitrectomy are minimal.

In addition, this procedure may introduce an increased risk of endophthalmitis.

Hence it may be possible and perhaps beneficial to administer this regimen via an intravitreal injection with a 30-gauge needle in an office-based setting. Before the present case series, we used a light dose for PDT that was 50% of the standard dose. In the present case series, however, we used an 84% light dose because we found that the 50% light dose was not completely effective in some patients (data on file). (We note that the 50% light dose is different from the 50% reduced fluence rate used in other studies.) We also found that a few patients (18 patients, 17.3%) had edema remaining after the triple therapy; these patients received an additional intravitreal injection of bevacizumab. Five patients were retreated with triple therapy due to recurrence of CNV activity. Other optimizations, such as changing the doses or type of steroid and anti-VEGF, or adjusting the timing of intravitreal injections with regard to verteporfin PDT, may result in better outcomes. Such optimizing of treatment regimen corresponds with the recent concept of personalized medicine, as well as acknowledges the differences between sound clinical judgment and the rigorous evidence required for drug approval.

The present case series, despite its limitations, indicates that combination therapy may offer, in a sustained manner after one cycle of therapy, functional VA benefit comparable to that observed with continued monthly anti-VEGF therapy. Our investigations will continue to further define such durability of benefit.

Conclusions

In most of the patients with CNV due to AMD, triple therapy with verteporfin PDT, bevacizumab, and dexamethasone results in significant and sustained VA improvement with only one cycle of treatment (occasionsly complemented by an additional intravitreal injection of bevacizumab). Such therapy offers VA benefit, a good safety profile, low cost over time compared with therapies that must be continued indefinitely, such as anti-VEGF therapies, and convenience for patients.

Key words: age-related macular degeneration (AMD), anti-inflammatory, anti-VEGF, bevacizumab, choroidal neovascularization (CNV), dexamethasone, photodynamic therapy (PDT), reduced fluence, steroid, triple therapy, verteporfin.

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