Combination of Photodynamic Therapy and Intraocular Triamcinolone for Exudative Age-Related Macular Degeneration and Long-Term Chorioretinal Macular Atrophy

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Objective: To evaluate the long-term effect of intravitreal triamcinolone acetonide (IVT) treatment combined with photodynamic therapy (PDT) vs PDT alone for neovascular age-related macular degeneration.

Methods: Prospective randomized study. Eighty-four patients were enrolled to receive PDT (n=41) or IVT treatment followed by PDT (n=43) within approximately a 7- to 15-day interval. All patients were naive to treatment. At baseline and each follow-up visit at 3, 6, 12, and 24 months, measurement of best-corrected visual acuity (VA), fluorescein angiography, indocyanine green angiography, and optical coherence tomography were performed. Mean changes in VA and retreatment rate were considered as primary outcome indicators. Analysis of vascular choroidal changes documented by indocyanine green angiography and fundus autofluorescence measurements were also performed.

Results: Mean VA increased at 1 month of follow-up but decreased progressively by the 24-month point in both groups (P=.74). The retreatment rate was significantly lower (P<.001) in the combined therapy group. Choroidal hypoperfusion/nonperfusion (P<.001) and areas with decreased/absent fundus autofluorescence within the PDT spot area were significantly greater with combined therapy (P<.001).

Conclusions: Combination IVT treatment with PDT seemed to be more effective for managing neovascular age-related macular degeneration, but long-term analysis failed to demonstrate functional benefits.

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Among the variety of paradigms implicated in the pathogenesis of choroidal neovascularization (CNV), a recent point of interest has been inflammatory and angiogenic components. The mechanisms involved include cell-mediated inflammation, leukocyte adhesion and extravasation, angiogenesis, and matrix deposition and remodeling. In particular, vascular endothelial growth factor (VEGF) and pigment epithelium–derived factor are important mediators in the development and growth of CNV, with VEGF having a stimulatory effect on vascular exudation and neovascularization and pigment epithelium–derived factor, an inhibitory effect. Throughout the last decade, verteporfin photodynamic therapy (PDT) has received widespread support as a treatment for subfoveal neovascular age-related macular degeneration (AMD). It consists of a selective photothermolysis of the CNV triggered by local photoactivation of the drug verteporfin administered intravenously, with subsequent production of free radicals and other oxidative metabolites leading to modifications of the exposed endothelial cells and causing selective thrombosis. Major limitations of this technique are (1) high tendency of the new vessels to recur within 3 months, requiring multiple treatments, and (2) modest visual advantages compared with the natural course of the disease. Recent studies have also shown that PDT may itself induce the release of several potent immune system mediators, including components of the complement and clotting cascades, acute phase proteins, proteinases, peroxidases, cytokines, and growth factors. Since this immune response contributes to the regrowth of the neovascularuare, a combination of PDT with pharmacologic adjuncts (anti-VEGF drugs) has been explored as a means of improving the efficacy of neovessel ablation. In particular, steroids may have a potential synergistic effect when combined with PDT. They have antiinflammatory, antiangiogenic, antifibrotic, and antipermeability properties. Steroids stabilize the blood-retinal barrier, hence facilitating exudate reabsorption and down-
regulation of inflammation following PDT. These considerations represent the rationale for the development of a combined strategy aimed at reducing post-PDT VEGF upregulation, thereby prolonging the photothermolysis triggered by PDT and reducing the stimulus for recurrences.

A number of pilot studies have investigated the use of verteporfin PDT in combination with intravitreal triamcinolone acetonide (IVT) treatment in patients with subfoveal or juxtapfoveal CNV. All of these studies have reported favorable visual acuity (VA) outcomes and were associated with a cessation of leakage on fluorescein angiography (FA). The combination was well tolerated, with treatable adverse effects, such as cataract progression and a transient increase in intraocular pressure (IOP). One might speculate that more recent and better-tolerated anti-VEGF drugs, such as ranibizumab or bevacizumab, should substantiate these favorable results if combined with PDT. However, a possible risk of collateral adverse effects comes from the concept of the selectivity of PDT in closing only CNV, which may be significantly altered in combination strategies. Indeed, despite the potential benefits of adjunctive therapies, the long-term impact on the retinal pigment epithelium (RPE), neural structures, and choroidal circulation as yet remains unclear. The present study was designed to evaluate the long-term anatomical and functional effects of the combined treatment, IVT with PDT, vs PDT alone in a large group of patients with exudative AMD. We used a prospective, randomized, unmasked parallel-group design to compare both treatment regimens in patients with all types of CNV.

METHODS

A consecutive series of 84 white patients (84 eyes), 59 women (mean [SD] age, 67.6 [11.6] years) and 25 men (mean [SD] age, 73.6 [9.1] years), with clinical and angiographic evidence of CNV secondary to AMD were enrolled and randomly assigned either to combined IVT treatment and PDT (IVT-PDT group) or to the PDT-only regimen (PDT-only group). All patients signed a comprehensive consent form before entering the study. The study was approved by the University of Padova Human Research Ethics Committee and was performed in accordance with the tenets of the Declaration of Helsinki. According to the angiographic features of the CNV, patients of both groups were further divided into predominantly classic CNV, minimally classic or not classic CNV, and retinal angiomatosus proliferation. Patients with any previous treatment of the CNV or other confounding ocular disease, such as diabetic retinopathy or retinal vein thrombosis, were excluded from this study. Patients with CNV secondary to pathological myopia, idiopathic or inflammatory CNV, or CNV secondary to angiod streaks were also excluded. Forty-three patients (IVT-PDT group) received an injection of IVT (Kenalog; Bristol-Myers Squibb, New York, New York) 7 to 13 days before PDT. Forty-one patients who received only PDT (PDT-only group) were used as controls. In both groups, PDT with verteporfin (Visudyne; Novartis AG, Basel, Switzerland) was performed according to standard parameters, as described previously by the TAP Study. Each patient in the IVT-PDT group received an intravitreal injection of 0.1 mL of 4 mg of triamcinolone acetonide (TA) under sterile conditions in an operating room following pericocular preparation with 10% povidone/iodine (Betadine; Meda Pharma, Solna, Sweden) and a conjunctival flush with 3% povidone/iodine. All injections were applied inferotemporally at 4 mm from the limbus. Within 15 days (±3 days) after injection, they also received PDT with verteporfin. In the IVT-PDT group, topical antibiotics were given from 3 days before to 4 days after the injection of TA. Intraocular pressure was monitored 1 and 7 days after injection and monthly thereafter. Only those patients with an IOP higher than 24 mm Hg also received topical medication. Both groups were followed up for at least 24 months after the first treatment. At baseline, each patient underwent best-corrected VA (logMAR [logarithm of the minimum angle of resolution]) measurement with Early Treatment Diabetic Retinopathy Study charts, Goldman applanation tonometry, ophthalmoscopic examination, FA, indocyanine green (ICG) angiography with a scanning laser ophthalmoscope (Heidelberg Retina Angiograph HRA II; Heidelberg Engineering, Heidelberg, Germany), and optical coherence tomography (OCT) (Stratus OCT 3; Zeiss Jena GmbH, Jena, Germany). These examinations were repeated at 3, 6, 12, and 24 months. Fluorescein and ICG angiograms and OCT were performed to estimate the activity of the CNV and were independently (and jointly, in the case of discrepancy) evaluated by 2 expert readers (S.P. and G.L.G.). From the third month on, whenever FA showed leakage and/or increased CNV size and an increase of at least 100 µm of the retinal thickness due to macular edema, subretinal fluid, or RPE detachment, PDT was repeated (additional treatments occurred no sooner than 3 months ±15 days) after the last PDT session. To avoid adverse effects related to possible overdosing, IVT treatment was repeated after a minimum 6-month interval, when needed. Main outcome variables were best-corrected VA at baseline and at 1, 3, 6, 12, and 24 months, number of retreatments with PDT, and adverse events. Indocyanine green angiography was also performed to investigate the choroidal vasculature within the treatment area and the patency of CNV. Single 30° images were taken in rapid sequence during the first minute after ICG infusion and subsequently during late phases at 2, 5, 10, and 20 minutes. Confoocal ICG images from early and late series were analyzed prior to and at 3, 6, 12, and 24 months after treatment in all patients of both groups. Closure of 1 or more choroidal vessels of the Sattler and Haller layers within the PDT-irradiated area, as investigated by mid-phase ICG angiography, was considered evidence of choroidal hypoperfusion. At baseline and at the 24-month point, fundus autofluorescence (FAF) images, obtained with the Heidelberg Retinal Angiograph HRA II, were also analyzed. The Heidelberg Retinal Angiograph HRA II uses an argon blue laser light with a wavelength of 488 nm for excitation and a barrier filter with a cutoff at 500 nm to record FAF. Fundus autofluorescence images were produced using a 30° field-of-view mode. A series of digital images obtained and saved from each eye were averaged to reduce noise in order to produce the final FAF image. The distribution of FAF was evaluated at the site of the CNV, around the CNV, and outside the area affected by the CNV.

Comparisons of the 2 treatment groups were performed by t test or x2 test when quantitative or qualitative variables were analyzed, respectively. Time profiles of VA observed in the 2 groups were compared by a 2-way analysis of variance (ANOVA) for repeated measures (group per time), where “group” and “time” were considered the between- and within-factor, respectively. The Bonferroni test was used for post hoc multiple comparisons. In all statistical analyses, P < .05 was considered statistically significant. All analyses were performed using SAS version 9.1.3.
needed only 1 treatment, and 8 (18.6%) received 2 or more treatments (Table 2). Among those needing retreatment, 4 (9.3%) had a recurrence that was retreated, 1 with another combined treatment and 3 with PDT alone because of the patients’ refusal to receive further injections. In the PDT-only group, 4 patients (9.8%) received 2 treatments, 18 patients (43.9%) received 3 treatments, 9 patients (21.9%) received 4 treatments, and 10 patients (24.4%) received 5 or more treatments (χ² test, P < .001).

VISUAL ACUITY OUTCOMES

At baseline, mean (SD) best-corrected VA was 0.69 (0.47) in the IVT-PDT group and 0.69 (0.16) in the PDT-only group. Visual acuity increased at 1 month of follow-up (0.51 [0.38] and 0.65 [0.18] in the IVT-PDT and PDT-only groups, respectively) and then progressively decreased in both groups. Visual acuity worsened more rapidly in the PDT-only group, with values significantly better in the IVT-PDT group at 3, 6, and 12 months. However, at the 24-month point, the IVT-PDT group showed a significant worsening, reaching values statistically similar to the PDT-only group (Figure 1).

CHOROIDAL VASCULATURE MODIFICATIONS

Choroidal hypoperfusion was identified by ICG angiography within the treated area as closure of 1 or more major or midsize choroidal vessels. At 1 month of follow-up, all patients in the IVT-PDT group and 33 of 41 patients (80.5%) in the PDT-only group showed evidence of choroidal hypoperfusion within the treated area (Table 3). However, commencing 3 months post-PDT, a progressive reperfusion was observed in most of the PDT-only patients, while at the end of the follow-up, the majority of patients in the IVT-PDT group showed persistent closure of some choroidal vessels (Figure 2 and Figure 3).

OCT AND FA

A significant reduction of retinal thickness measured by OCT was found in the IVT-PDT group (P < .005). This parameter showed a reduction from a mean (SD) value of 354 (116.2) µm at baseline to 165.2 (40.7) µm at the 24-month follow-up in the IVT-PDT group and from 362.0 (122.7) µm at baseline to 240.2 (146.3) µm at the 24-month follow-up in the PDT-only group. The presence of leakage, evaluated during the late phase of FA, was considered an active index lesion (Figure 4). A statistically significant difference became evident by the third month of follow-up and remained significant in favor of the IVT-PDT group throughout the follow-up (Table 4).

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Modification of FAF within the PDT spot area was also analyzed. Only decreased/absent FAF areas, which actually indicate atrophy of the RPE, were considered. At the end of the follow-up, 38 IVT-PDT patients (88.3%) had areas of decreased FAF within the PDT spot area that were not evident at baseline. This was evident in only 5 patients (12.1%) in the control group (χ² test, P < .001) (Figure 5 and Figure 6). Data show no correlation between decreased FAF and number of IVT-PDT treatments. In the IVT-PDT group, RPE atrophy significantly correlated with VA and choroidal closure (ANOVA: F = 3.74; P = .03 and F = 219.69; P < .001, respectively).

COMPLICATIONS

No cases of endophthalmitis or retinal detachment were reported in the IVT-PDT group. At baseline, 1 patient in the IVT-PDT group and 2 in the PDT-only group had pseudophakia. During the study, 21 patients (48.8%) in the IVT-PDT group needed cataract surgery vs 8 patients (19.5%) in the PDT-only group. Forty percent of patients who received only 1 IVT-PDT treatment developed cataracts vs 87.5% of patients who underwent 2 or more treatments (Fisher exact test, P = .02). In patients who received IVT treatment, 4 (9.3%) had an IOP higher than 24 mm Hg, with the maximum values at the 3-month follow-up (range, 24–27 mm Hg). These patients were successfully treated with adjunctive therapy.

COMMENT

Progressive understanding of angiogenesis mechanisms has led to new therapeutic strategies, such as selective treatment for CNV with PDT or intraocular injection of anti-VEGF drugs. Two placebo-controlled studies, Treatment of Age-related Macular Degeneration with Photodynamic Therapy (TAP) and Verteورporfin in Photodynamic Therapy (VIP), have shown some efficacy of PDT in the treatment of predominantly classic CNV and occult CNV, respectively.7,15 However, frequent persistence and recurrence of CNV, which require multiple treatments and are associated with poor long-term visual outcomes, are major limitations.13,33 Recent studies
have demonstrated that PDT causes selective photothermosis but it also produces inflammatory effects with delivery of local proangiogenic factors. Upregulation of VEGF may explain the high recurrence rate of CNV (nearly 98%) within 3 months following PDT. It has also been demonstrated that PDT is followed by local hypoxia and inflammation. The combination of anti-inflammatory agents, such as steroids, with PDT may therefore minimize or even prevent macular edema and regrowth of CNV. Tramcinolone acetonide interferes with these events, eventually preventing VEGF upregulation after PDT. Many studies have demonstrated the efficacy of combining TA with PDT to stabilize the effect of PDT. Spaide et al originally reported a 12-month follow-up of 26 patients with CNV who underwent combined therapy with PDT and an injection of 4 mg of TA. They reported an average improvement of 2.5 lines with a mean 1.24 combined treatments. Augustin and Schmidt-Erfurth reported a 24-month follow-up outcome of 41 patients treated with PDT followed by an injection of 25 mg of TA. The percentage of patients who improved 3 or more lines was 29.3% at 12 months and 31.7% at 24 months. Cumulative data from other clinical studies suggest that the combination of TA with PDT may yield better visual outcomes, less CNV recurrence, and less need for retreatments.

Our study confirms that the combination of TA with PDT significantly reduces the number of PDT retreatments needed to obtain permanent inactivation of CNV, as confirmed by FA and OCT. However, analysis of long-term visual outcomes reveals that after an initial significant benefit that lasts for at least 1 year, the combination of PDT with TA is associated with progressive visual decline during the second year of follow-up. These data are partially in contrast with those recently reported by Ruiz-Moreno et al, who showed long-term visual benefits in patients who received PDT and TA compared with control patients with AMD who underwent only PDT. However, these differences could be explained by the limited sample size (30 study eyes and 15 controls) of that study, the absence of randomization, and the relatively worse baseline VA of the patients who received only PDT.

In our study, patients who received combined therapy showed a 24-month VA comparable with the visual outcomes of control patients who underwent PDT only. Both groups experienced a significant deterioration of VA compared with baseline. In the combined therapy group, reduction of visual function was associated with atrophic changes of the central chorioretina, as shown by retinal thinning on OCT, significant rarefaction of choroidal vessels within the treatment area, and the presence of reduced/absent macular FAF.

Analysis of retinal FAF has recently been introduced as a tool to clinically investigate changes of the RPE related to the accumulation of the retinal age pigment lipofuscin. Despite little being known of the pathogenesis and clinical importance of areas of increased FAF, it is clear that absent FAF reflects strong atrophic changes of the RPE and photoreceptors. In our study, areas of absent macular FAF were associated with local rarefaction of choroidal vasculature and inversely correlated with VA. Interestingly, although choroidal vascular changes could be documented.
in most patients within the IVT-PDT group soon after PDT and throughout the follow-up, this was associated with a VA deterioration that was documented only at the 2-year follow-up, long after the last IVT-PDT treatment. This may be explained by the survival capabilities of neuronal structures of the retina, even in the presence of a relatively damaged choroidal vasculature.

With the widespread use of antiangiogenic drugs, such as bevacizumab or ranibizumab, our observations on TA use may appear obsolete. However, 2 major points can be
extrapolated from our study. First, when considering new therapies for exudative AMD, only long-term (at least 2 years) observations may prove the real efficacy on visual function. Some drugs, such as TA, may temporarily improve VA but are not associated with stable benefits. Second, atrophic changes that we have reported with TA may be even more noticeable with specific anti-VEGF drugs.

Ultrastructural studies have provided evidence that choriocapillaris undergo continuous remodeling in the adult, with large vessels showing fenestrations at the side facing the RPE. In mutant mice, it has been demonstrated that deficiency in VEGF expression in the RPE results in absence of the choroid. Other studies suggest that VEGF signaling from the RPE is involved not only in choroidal vessel formation, but also in the maintenance of the choriocapillaris. Absence of VEGF causes secondary atrophy of the choriocapillaris and results in loss of endothelial cell fenestrations. Intraocular injections of bevacizumab in primates produce ultrastructural changes in the choriocapillaris with significant reduction of cell fenestrations. The choriocapillaris is essential for the survival of the neurons of the outer retina, providing these cells with oxygen and nutrients via the intercellular junctions and endothelial cell fenestrations. Photodynamic therapy–mediated photothermolysis is dose dependent and temporarily closes targeted CNV along with most of the small and medium-sized choroidal vessels within the treated area. Upregulation of VEGF, which takes place shortly after PDT, allows almost complete recanalization of the occluded vessels. Thus, combining PDT with an anti-VEGF drug may not only prevent CNV recurrences but may also significantly affect the restoration of a normal choroidal vasculature within the treated area. Prolonged inhibition of VEGF upregulation after IVT-PDT may explain the atrophic changes at 2 years of follow-up, which are responsible for the poor visual outcomes we have reported in this study.

**Table 4. Choroidal Neovascularization With Angiographic Leakage at Baseline and 1-, 3-, 6-, 12-, and 24-Month Follow-up Visits**

<table>
<thead>
<tr>
<th>No. (% of Patients)</th>
<th>Baseline</th>
<th>1 mo (13.9)</th>
<th>3 mo (4.6)</th>
<th>6 mo (4.6)</th>
<th>12 mo (9.3)</th>
<th>24 mo (4.6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVT-PDT</td>
<td>43 (100.0)</td>
<td>6 (13.9)</td>
<td>2 (4.6)</td>
<td>2 (4.6)</td>
<td>4 (9.3)</td>
<td>2 (4.6)</td>
</tr>
<tr>
<td>PDT only</td>
<td>41 (100.0)</td>
<td>13 (31.7)</td>
<td>37 (90.2)</td>
<td>27 (65.9)</td>
<td>21 (51.2)</td>
<td>21 (51.2)</td>
</tr>
</tbody>
</table>

Abbreviations: See Table 2. 

\( P < .05 \) was considered statistically significant.
Despite this current study having some limitations, such as the single-center enrollment and the lack of a sham injection in the PDT-only group, our observations confirm the synergistic effects of PDT and TA administration. If these data were to be extrapolated to combined therapies with new antiangiogenic drugs, care should be taken when drawing final conclusions from clinical trials of less than 2 years' duration. Also, attention should be dedicated to optimize

Figure 5. Patient who received intravitreal triamcinolone acetonide injection with photodynamic therapy. A-C, Baseline late fluorescein angiography (A) and early (B) and late (C) indocyanine green (ICG) angiography showing the presence of a leaking choroidal neovascularization. D, Horizontal scan with optical coherence tomography at baseline shows retinal edema and subretinal detachment. E, At the 24-month follow-up, the retina is completely flat, and atrophic changes of the deep layers are visible on horizontal optical coherence tomography. F and G, Late-phase fluorescein angiography at 24 months (F) shows only mild staining of the chorioretinal scar, while early ICG angiography shows rarefaction of the choroidal vasculature within the treated area (G). H, In the late phases of ICG angiography, a halo of persistent hypofluorescence is still visible. I, Autofluorescence, which should reflect the presence of viable retinal pigment epithelial cells, demonstrates the presence of an area of decreased/absent fundus autofluorescence corresponding to the irradiated area, which confirms the atrophic changes in the same area.

Figure 6. Patient who received only photodynamic therapy. A-C, Baseline late fluorescein angiography (A) and early (B) and late (C) indocyanine green angiography show angiographic signs of a minimally classic lesion. The patient was treated with 4 sequential sessions of photodynamic therapy. D and E, Horizontal scan with optical coherence tomography at baseline (D) shows sensory-retina edema that persists at the 24-month follow-up (an elevation of the retinal pigment epithelium is also observed [E]). F, The 24-month late phase of fluorescein angiography shows intraretinal and subretinal leakage. G and H, On indocyanine green angiography, choroidal neovascularization is still visible (G), although associated with reduced dye staining (H). I, Autofluorescence at 24 months shows only minimal changes within the treated area.
dosing to prevent occlusion of larger choroidal vessels and consequent atrophy of macular neuroretina.

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REFERENCES