SCIENTIFIC REPORT

Histopathological findings in postmortem eyes after photodynamic therapy for choroidal neovascularisation in age-related macular degeneration: report of two cases

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Background: To report the histopathological findings after photodynamic therapy (PDT) in eyes obtained postmortem with choroidal neovascularisation (CNV) secondary to age-related macular degeneration (AMD).

Methods: Two eyes were obtained postmortem from two patients with CNV secondary to AMD. Both of the patients had been treated with PDT. Serial sections through the posterior poles were obtained and stained with haematoxylin-eosin, periodic acid-Schiff, Masson trichrome or phosphotungstic acid haematoxylin (PTAH). Two-dimensional reconstructions were prepared and compared with fluorescein angiograms.

Results: The interval between PDT and death was 3 months and 17 months in each patient, respectively. Light-microscopic examination showed that CNV enveloped with retinal pigment epithelium (RPE) in both eyes. The average size of the CNV was 550×280 μm. One eye had combined (subRPE/subretinal) growth pattern CNV, and the other eye had both type I (subRPE) and combined growth pattern CNV. All specimens contained fibrous proliferation and patent vascular channels within the CNV, and there was no thrombus formation within the vascular channels. No apparent abnormalities in the choroid were observed by light microscopy.

Conclusions: Although involution with fibrous tissue proliferation occurred, PDT did not result in permanent occlusion of the vascular channels in the CNV. Our findings indicate that PDT may accelerate involution of CNV, thus limiting its size and preserving photoreceptors.

Age-related macular degeneration (AMD) is the leading cause of severe vision loss in people older than age 65 years in the Western world.[1 2] The main cause for vision loss is the development of subfoveal choroidal neovascularisation (CNV).[3] Photodynamic therapy (PDT) using verteporfin has emerged as an important method for treating certain subtypes of subfoveal CNV in patients with AMD.

PDT with verteporfin was shown to induce selective destruction of vascular endothelial cells within the choriocapillaris layer when used at standard doses in a laser-induced model of CNV.[4] Several histopathological studies of surgically excised CNV after PDT have been reported.[5–9] There has been one case report of the clinicopathological findings of CNV after PDT in a postmortem eye from a patient with AMD.[10] In that study, a 77-year-old man with AMD and subfoveal CNV developed a recurrent CNV 8 months after macular translocation surgery and underwent PDT. There was a short-term effect of the treatment, which was 2 weeks after PDT.

Since PDT has a temporary vaso-occlusive effect and patients need repeated treatments, histopathological evaluation of eyes obtained postmortem with a long interval after PDT is important. Herein, we report the histopathological findings in two eyes obtained postmortem after PDT for CNV from AMD. This study provides information regarding the long-term histopathological effects of PDT.

MATERIALS AND METHODS

Two eyes were obtained postmortem from two patients with CNV secondary to AMD with a past medical history of PDT treatment. The eyes were obtained from 2 eyebanks (Lions Medical Eye Bank, Norfolk, VA and Lions Eye Bank of Oregon, Portland, OR) through the PDT Pathology Center Eye Donation Program in Emory Eye Center. This study was approved by the Emory University Institutional Review Board (IRB). For light-microscopic processing, the eyes were fixed in 10% neutral-buffered formalin, dehydrated in increasing concentrations of alcohol, cleared in xylene and embedded in paraffin. Serial 7-μm-thick sections through the posterior pole were made using a microtome (Finesse, Thermo Shandon, Astmore, UK), numbered and sequentially stained with haematoxylin-eosin, periodic acid-Schiff, Masson trichrome, and Prussian blue. For the evaluation of thrombi formation within the CNV, phosphotungstic acid haematoxylin (PTAH) stain was performed. The slides were sequentially examined using a microscope (Olympus BH-2, Olympus, Tokyo) with a standard reticule, and a two-dimensional map was constructed as described previously.[11] The size of the CNV was measured, and the CNV growth pattern was described as subRPE (type 1), subretinal (type 2) or combined (subRPE and subretinal).[12] When available, the findings from the fluorescein angiograms were compared with the histopathological findings.

RESULTS

Two eyes from the patients who had a documented history of previous PDT treatment were received and examined. The clinicopathological features of the cases are summarised as follows.

Case 1

An 83-year-old woman with AMD and bilateral cataract extractions with posterior chamber intraocular lenses (IOLs) developed subfoveal CNV in her right eye. She underwent PDT for the subfoveal CNV with an adjuvant intravitreal triamcinolone injection (4 mg/0.1 ml) in August 2003. She died of a subdural haemorrhage 3 months after the PDT.

Gross examination of the right eye showed retinal haemorrhages in the macula (fig 1A). Histological examination showed areas of intraretinal haemorrhages in the posterior pole and a combined growth pattern (subRPE & subretinal) of CNV under

Abbreviations: AMD, age-related macular degeneration; CNV, choroidal neovascularisation; IOL, intraocular lens; PDT, photodynamic therapy; PTAH, phosphotungstic acid haematoxylin; RPE, retinal pigment epithelium

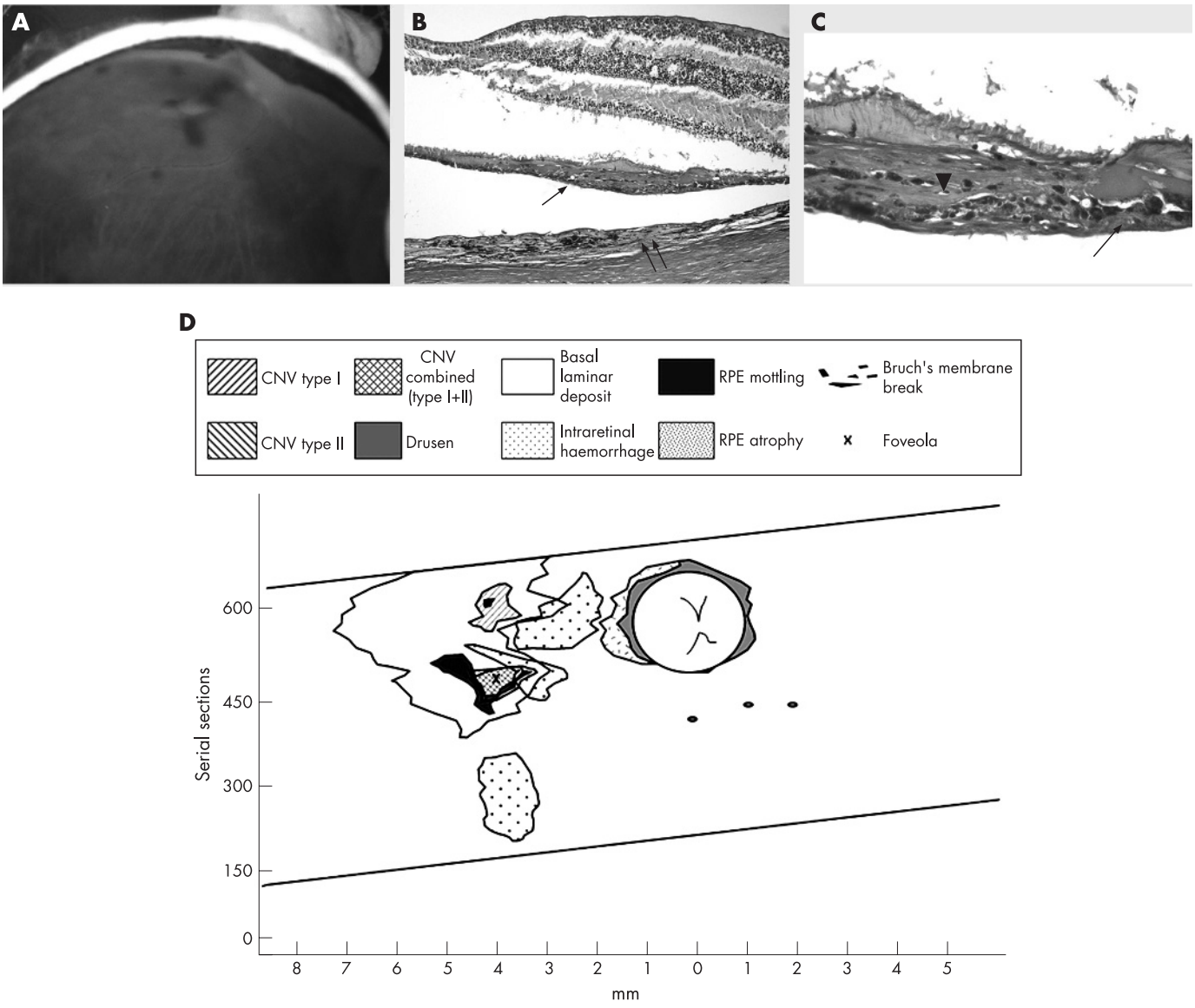

Figure 1 Case 1. (A) Gross photograph of the right eye. The macula contains retinal haemorrhages. (B) A combined growth pattern CNV (subRPE/subretinal) (arrow) is present under the fovea. The membrane measures 400 μm in maximum width, and 70 μm in maximum thickness (periodic acid-Schiff, original magnification ×100). Focal loss/closure of the choriocapillaris is present (double arrows). (C) The membrane contains fibrous tissue and patent blood vessels (arrowhead). Basal laminar deposits under the RPE are present within the membrane (arrow). (PAS, ×400) (D) Two-dimensional reconstruction showing areas of intraretinal haemorrhage in the macula and a small area of combined growth pattern CNV in the fovea. CNV type 1, sub-RPE growth pattern; CNV type 2, subretinal growth pattern; RPE, retinal pigment epithelium.

the fovea (fig 1B), measuring 400 μm in maximum dimension and 70 μm in maximum thickness. Fibrous tissue and patent vascular channels were present within the CNV, and the PTAH stain was negative. There was atrophy of the photoreceptors overlying the combined type CNV. Focal loss and/or closure of the choriocapillaris was present in the macular region. Basal laminar deposits were present under the RPE (fig 1C). A small subRPE (type 1) growth pattern of CNV was found superior to the macula. Two-dimensional reconstruction showed areas of intraretinal haemorrhage in the macula and an area of combined growth pattern CNV in the fovea (fig 1D).

Case 2

A 67-year-old man with AMD developed subfoveal CNV in his left eye. In the past, he had received several laser treatments for central serous chorioretinopathy in his right eye. He underwent PDT for the subfoveal CNV in the left eye in March 2003. A fluorescein angiogram of the left eye taken before the PDT showed subfoveal classic type CNV (fig 2A). The classic CNV in the fovea increased in size, even after PDT on the follow-up fluorescein angiograms, and was surrounded by a stippled pattern of hyperfluorescence, which was interpreted as an area of occult CNV, that is predominantly classic CNV arising in occult CNV. In spite of the increasing size of CNV, the patient did not receive additional PDT. He died of a myocardial infarction 17 months after the PDT.

Gross examination of the left eye showed no haemorrhage or fibrous scar in the macular area (fig 2B). Histological examination showed a combined growth pattern of CNV in the subfoveal area, which measured 700 μm in greatest width and 170 μm in thickness (fig 2C). A reflected layer of RPE was present within the membrane with fibrous tissue, and patent vascular channels were identified (fig 2E). The combined CNV was surrounded by a thin area of subRPE CNV (fig 2F). The RPE overlying the CNV was attenuated immediately surrounding the combined growth pattern CNV, and the RPE was intact,

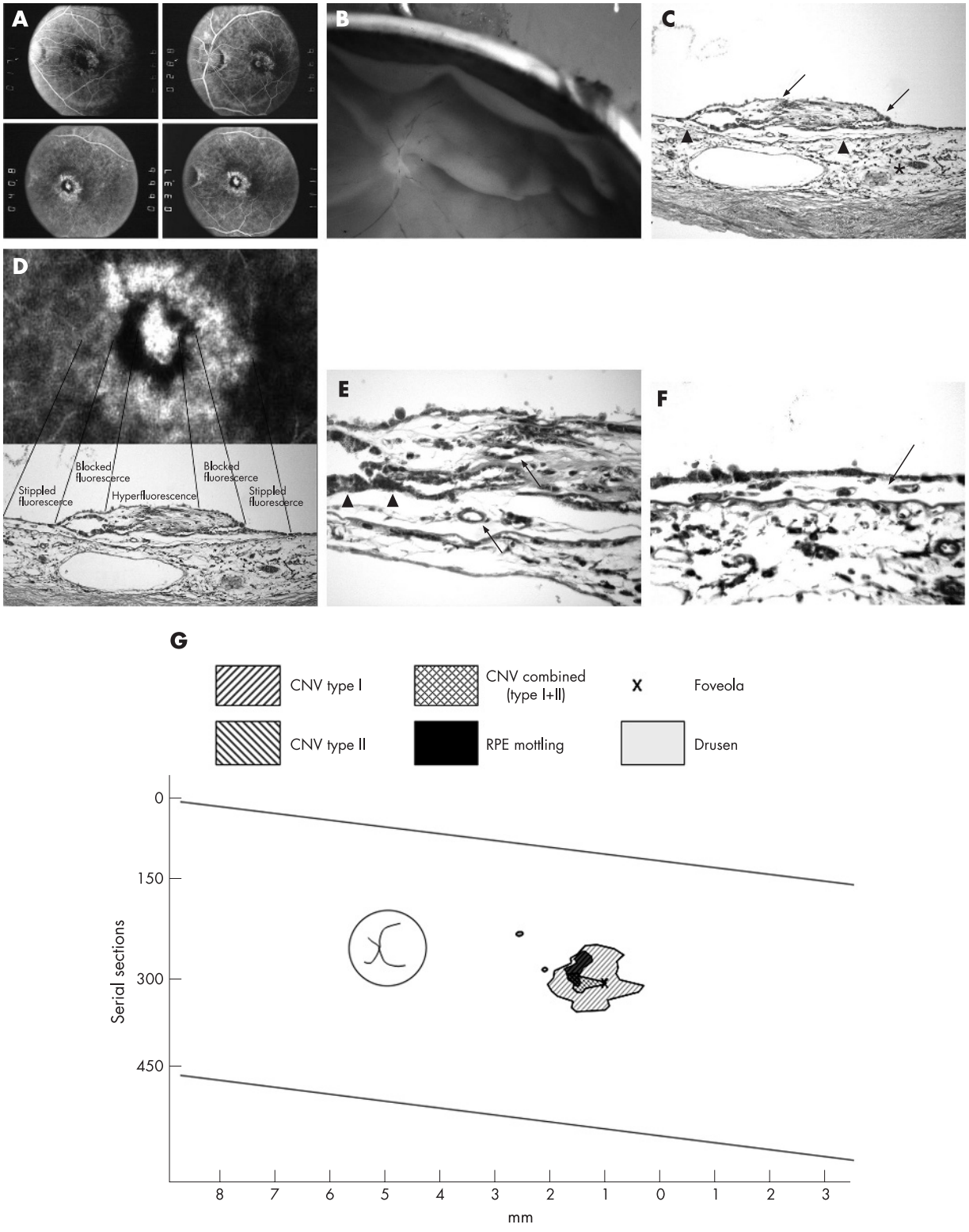

Figure 2 Case 2. (A) Fluorescein angiography of the left eye obtained in March 2003 (top left), May 2003 (top right), September 2003 (bottom left), and December 2003 (bottom right). The classic angiographic pattern CNV increased in size despite PDT in March 2003. The patient died in July 2004. (B) Gross photograph of the left eye. No haemorrhages or scarring in the macula are noted. (C) Combined growth pattern CNV located in the subfoveal area. The CNV has both a subRPE component (arrowheads) and subretinal component (arrows). The membrane measures 700 μm in maximum width and 170 μm in maximum thickness. The overlying RPE is attenuated. Focal loss/closure of the choriocapillaris is present (asterisk). (Masson trichrome, original magnification ×100). (D) Diagram showing how the histology in (C) corresponds with angiogram in (A), bottom-right subset. The combined pattern of CNV corresponds to the hyperfluorescence in the angiogram, and the peripheral subRPE growth pattern CNV with attenuated RPE corresponds to the stippled fluorescence. The blocked fluorescence in the angiogram corresponds to double layering or overlapping RPE. (E) CNV consisting of a reflected layer of RPE (arrowheads), fibrous tissue and patent vascular channels (arrows). (Masson trichrome, ×400). (F) Thin subRPE (type 1) growth pattern CNV (arrow), surrounding the outer rim seen angiographically. Note the focal hypertrophy and attenuation of the RPE overlying this area of thin CNV. This area is not apparent in the fluorescein angiogram (Masson trichrome, ×400). (G) Two-dimensional reconstruction of the histological findings, showing that the angiographic classic CNV corresponds to the combined growth pattern CNV. The immediately surrounding angiographic occult CNV (stippled pattern) correlates with CNV covered with attenuated RPE. CNV type 1, subRPE growth pattern; CNV type 2, subretinal growth pattern; CNV combined, subRPE and subretinal growth pattern; RPE, retinal pigment epithelium.

overlying the more peripherally located CNV. There was atrophy of the photoreceptor layer overlying the attenuated RPE and the combined type CNV. The PTAH stain was negative. Focal loss and/or closure of the choriocapillaris was present in the macular region. Comparison of two-dimensional reconstruction with the fluorescein angiographic findings showed that the angiographic classic CNV corresponded to the combined growth pattern CNV. The immediately surrounding angiographic occult (stippled pattern) CNV correlated with CNV covered with attenuated RPE. The more peripheral CNV was not recognised angiographically and was covered by apparently normal RPE. These findings were topographically located in the two-dimensional reconstruction (fig 2G).

DISCUSSION

There are several previous studies regarding the histopathological findings after PDT, mostly from surgically excised CNV membranes. The report using a postmortem eye showed occluded vascular channels and PTAH-positive thrombi in vessels in the CNV 2 weeks after PDT.[10] In our current study, the vascular channels were patent in both specimens, and no thrombi were noted within the vessels at 3 months (case 1) and 17 months (case 2) after a single treatment of PDT. A report regarding the histopathological findings of surgically excised CNV 4 months after PDT revealed that there were patent vascular channels.[2] A clinicopathological study with eight submacular choroidal neovascular membranes obtained from 3 days to 152 days after PDT demonstrated that the CNV obtained 3 days after PDT showed partial vascular occlusion, which was not present in specimens obtained at longer intervals after PDT (29–152 days).[7] Ultrastructural examination revealed evidence of vascular damage in all the specimens obtained at longer intervals after PDT, and all the specimens had patent CNV. Considering this reopening or regrowth of vascular channels in CNV after PDT, the current retreatment interval of 3 months seems appropriate.

Transient choroidal ischaemia after PDT with verteporfin in AMD has been observed previously in human and animal studies.[13–15] Tzekov and colleagues demonstrated histopathological changes in monkeys 9 months after a single session of PDT. They included fibrovascular proliferation in the choriocapillaris associated with closure, and RPE degeneration and necrosis.[16] Another study showed perfusion defects in the choroid in patients receiving a single PDT. The defects started at 1 day and were still present at 3 months after PDT.[17] In our study, there were no light-microscopic abnormalities in the choroid (figs 1B and 2C), but the cellular and subcellular effects as well as the functional effects of long-term PDT cannot be completely ruled out. The clinical effect of the multiple sessions of PDT on the choroid warrants further histopathological evaluation.

Patent blood vessels were present in the CNV in our cases after a single session of PDT. CNV growth is a dynamic process over time, with initiation, inflammatory active, and involutional or inflammatory inactive stages.[12 18] RPE cells and macrophages interact with each other during the initiation stage of CNV by increased production of monocyte chemotactic protein by RPE.[18] Macrophages and RPE express VEGF in the active inflammatory stage, thus stimulating angiogenesis. This is followed by an inactive inflammatory (involutional) stage with decreased production of cytokine, especially VEGF, and increased amount of fibrosis as CNV matures.[18] A recent study showed that VEGF expression was significantly increased in RPE in CNV treated with PDT, shortly after the treatment.[19] The balance between VEGF and pigment epithelium derived factor (PEDF) was disturbed in the CNV membranes following PDT, resulting in an increased VEGF to PEDF ratio in the RPE and stroma.[20] This situation favours angiogenesis in the CNV. These findings are compatible with the persistence of patent blood vessels in the CNV after PDT in our cases, by recanalisation and/or angiogenesis.

The median size of surgically excised CNV was 1980×325 μm in patients with AMD enrolled in the Submacular Surgery Trials (SST), and 1800×395 μm in patients with predominantly haemorrhagic lesions attributed to AMD.[9] The CNV from our current cases 1 and 2 measured 400×70 μm and 700×170 μm, respectively, thus exhibiting a smaller size compared with the results from SST. Even though PDT does not result in the permanent closure of vascular channels in CNV, it appears to shorten the active stage of the CNV growth, thus accelerating the involutional stage.[12] This corresponds to a smaller CNV size and preservation of photoreceptors. Limitations of our study included the limited number of the cases (2 eyes), the lack of detailed clinical information of the patients and lack of ultrastructural information.

Our first case received a single intravitreal injection of triamcinolone with the PDT. High doses of intravitreal triamcinolone (more than 4 mg) have been shown to cause structural changes such as destruction of photoreceptor outer segments and migration of macrophage-like cells in the subretinal spaces in rabbits.[21] For the purposes of our study, the dose of 4 mg in the vitreous of a human eye is equivalent to 0.5 or 1 mg in the rabbit eyes, so it is assumed that the triamcinolone had no tissue effect by light microscopy.

In summary, although involution with fibrous tissue proliferation occurred, PDT did not result in permanent occlusion of vascular channels in CNV. However, PDT does appear to limit the size of CNV and accelerates CNV involution.

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