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SHORT COMMUNICATION

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Histological findings of a surgically excised myopic choroidal neovascular membrane after photodynamic therapy

A case report

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Abstract *Background:* The authors describe a myopic choroidal neovascular membrane excised 4 months after photodynamic therapy (PDT). *Methods:* A 68-year-old woman with classic choroidal neovascularization (CNV) due to pathologic myopia underwent PDT with verteporfin in the left eye. Four months after treatment a full-thickness macular hole was diagnosed in the same eye and the patient underwent vitrectomy with submacular membranectomy. The subfoveal membrane was studied by light microscopy and immunohistochemical techniques. *Results:* Light microscopy showed a thin fibrovascular membrane covered by residual retinal pigment epithelium. The membrane contained homogeneous matrix with small collagen bundles, fibroblasts and small blood vessels. The distribution of blood vessels was nonuniform: extravasated red blood cells, macrophages and other inflammatory elements were not present in the fibrous matrix. Endothelial cells were highlighted by CD34 immunostaining and did not show any significant alteration. There was no evidence of inflammatory cells or thrombosis inside vascular lumina. *Conclusions:* Histologic examination of the neovascular membrane showed features similar to those of surgically excised myopic CNV without PDT treatment. Our findings suggest that PDT-induced occlusion is temporary. Fluorescein leakage from CNV after a single PDT treatment can be considered as an sign of blood vessel regrowth or recanalization indicating that multiple treatments are necessary.

Introduction

Choroidal neovascularization (CNV) occurs in a variety of ocular diseases and is often accompanied by severe and irreversible loss of central vision [1, 9].

The etiopathogenesis of CNV is not yet well delineated; however, many histopathologic studies found a substantial similarity between cellular and extracellular components of choroidal neovascular membranes in different diseases presenting this complication [7]. This suggests that CNV represents a nonspecific wound repair-like response to a specific unknown stimulus [6].

This hypothesis is also supported by the fact that the common cellular and extracellular constituents of neovascular membranes are comparable to those found in granulation tissue [6, 7, 12].

After age-related macular degeneration (AMD), pathologic myopia is the most frequent ocular disease complicated by CNV [2].

Although CNV in myopia is self-limited, the visual outcome is fairly poor, particularly in patients over 50 years of age [22].

To date, laser photocoagulation is the only accepted treatment effective in limiting severe visual loss in selected cases of neovascular membranes located outside the foveal avascular zone or extended to the edge of the perifoveal capillary network [16].

Conversely, thermal laser treatment is not indicated for subfoveal myopic CNV, since full-thickness retinal

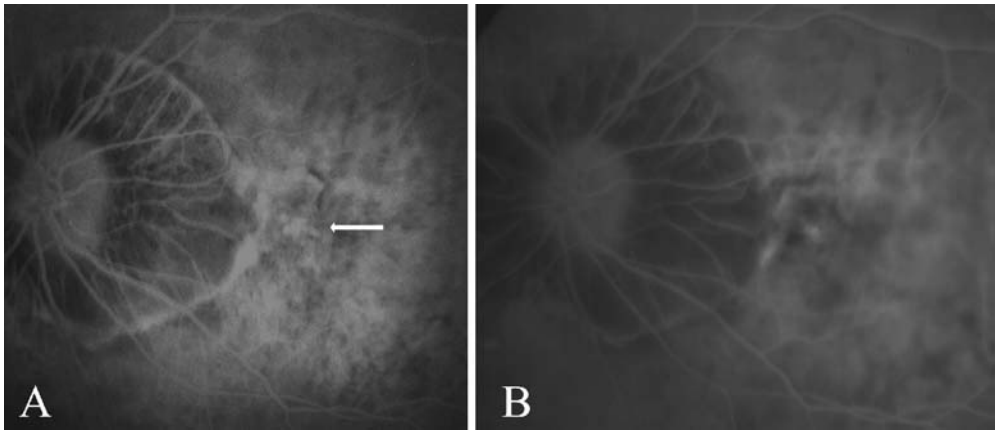

Fig. 1 **A** Fluorescein angiography shows focal hyperfluorescence with leakage due to a subfoveal choroidal neovascular membrane (*arrow*). **B** One month after verteporfin photodynamic therapy, fluorescein angiograms reveal sharply reduced hyperfluorescence of the neovascular membrane

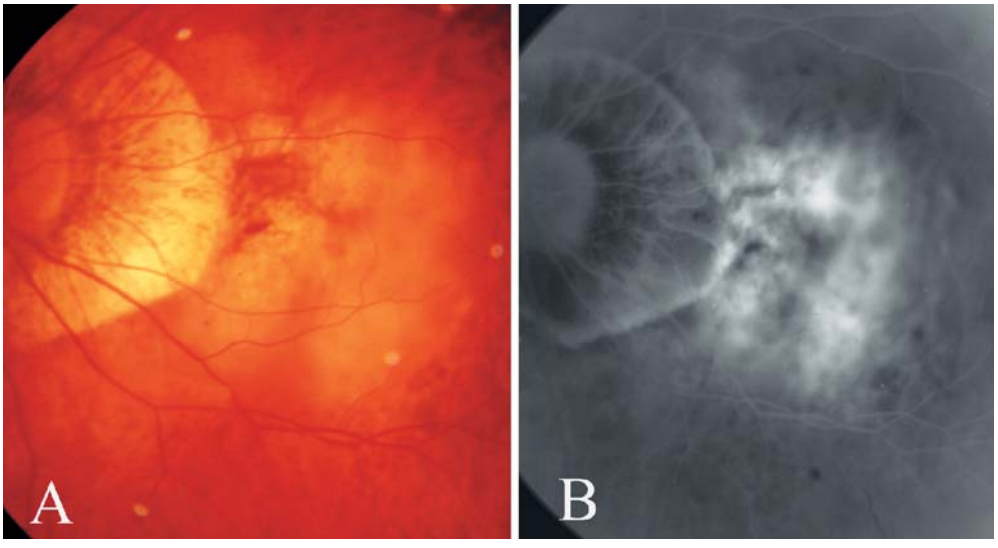

Fig. 2 **A** Color photograph of the left eye 4 months after PDT treatment shows posterior retinal detachment due to macular hole. **B** Fluorescein angiography at this time shows hyperfluorescence of CNV with leakage of the dye in the subretinal space

damage induced with laser photocoagulation can outweigh any treatment benefit [3].

Photodynamic therapy (PDT) is a promising therapeutic approach for the management of subfoveal CNV first used safely in AMD. Recently, randomized clinical trials have reported an increased chance of stabilizing or improving visual outcome also in patients affected by subfoveal CNV due to pathologic myopia [27]. Photochemical vascular occlusion induced by PDT was first documented in experimental animal models of CNV and confirmed by clinical studies that showed short-term (1–4 weeks) cessation of fluorescein leakage from new vessels [14].

The effects of PDT on human CNV have been documented in a few morphological studies in patients with AMD [4, 8, 20], whereas, to our knowledge, morphological features of surgically excised myopic CNV after PDT have been described only in two cases that have not shown improvement after PDT [15].

Material and methods

Fluorescein angiograms of a patient with pathologic myopia showed a classic subfoveal choroidal neovascular membrane in the left eye (Fig. 1A). One week later, the patient underwent PDT with verteporfin following the TAP Study Group guidelines [23]. After treatment the patient noticed a decrease in metamorphopsia and improvement of visual acuity. Fluorescein angiography revealed hyperfluorescence from the neovascular membrane in the early phase, with staining of CNV in the late phase (Fig. 1B). Four months after initial PDT therapy the patient complained of a significant deterioration of vision in the left eye. Fundus examination showed a posterior retinal detachment due to macular hole (Fig. 2A), and fluorescein angiography evidenced leakage from the CNV and pooling of the dye into the detached area of the sensory retina (Fig. 2B). A few days later the patient underwent a pars plana vitrectomy with internal limiting membrane peeling and surgical removal of neovascular membrane.

The surgical specimen underwent preliminary embedding in agar according to a recently described technique [25, 26]. In particular, the membrane tissue was distended on a slide and surrounded by fluid agar. Once the agar had become solid, the resulting block was fixed in 10% buffered formalin for 24 h and routinely processed to obtain serial 4-μm-thick sections, stained with

hematoxylin-eosin and Masson's trichrome method. An additional slide was immunostained with monoclonal antibody against CD34, using the LSAB peroxidase method.

Results

A 68-year-old woman affected by high myopia in both eyes was referred to our clinic because of metamorphopsia and blurred vision of recent onset in her left eye. Best-corrected visual acuity was 20/20 in the right eye and 20 /100 in the left eye, with −18- and −19-diopter correction respectively. The patient underwent a complete ocular examination. Anterior segment findings were unremarkable. Fundus examination and fluorescein angiography showed a classic subfoveal choroidal neovascular membrane in the left eye. One week later, the patient underwent PDT with verteporfin in the left eye. After PDT the patient noticed a decrease in metamorphopsia and improvement of visual acuity in the treated eye. One month after treatment the visual acuity was 20/40. Fluorescein angiography revealed hyperfluorescence from the neovascular membrane in the early phase, with staining of CNV in the late phase. Three months later the patient returned for follow-up fluorescein angiography. She complained of loss of vision in her left eye which had begun suddenly a few days previously. Visual acuity in the left eye was reduced to counting fingers. Slit-lamp biomicroscopic examination of the fundus disclosed a posterior retinal detachment due to macular hole. Fluorescein angiography evidenced hyperfluorescence of CNV with leakage during examination. In the late phase pooling of the dye into the detached area of the sensory retina was observed. Optical coherence tomography clearly displayed a full-thickness macular hole, an optically clear zone under the neurosensory retina for fluid accumulation and a hyperreflective tissue above the retinal pigment epithelium (RPE), consistent with type 2 neovascular membrane, according to the Gass classification of CNV.

Once obtained informed consent, the patient underwent surgical removal of neovascular membrane which was performed 4 months after PDT. Three months after surgery vision improved to 20/200, neuroretina appeared completely attached to RPE. Fluorescein angiography showed a small area of hyperfluorescence due to window defect caused by RPE atrophy in foveal area.

Pathologic findings

Histologic examination of the excised membrane showed a thin fibrovascular tissue covered by RPE and containing homogeneous matrix and small collagen bundles—more evident with trichrome stain—fibroblasts and small, irregularly shaped blood vessels (Figs. 3, 4).

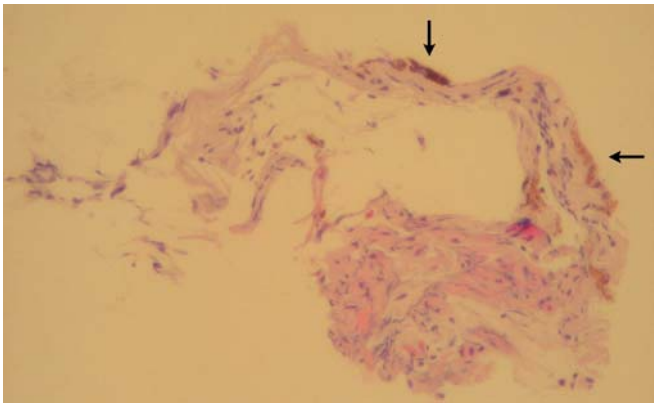

Fig. 3 General view of the neovascular membrane after PDT shows a thin fibrovascular tissue covered by residual retinal pigment epithelium (*arrows*) and containing hyaline matrix, fibroblasts and small blood vessels (hematoxylin and eosin, original magnification ×40)

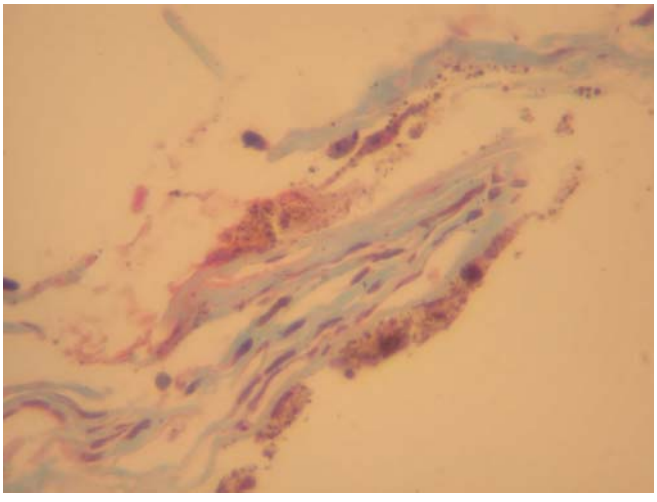

Fig. 4 Remnants of retinal pigment epithelium resting on small collagen bundles highlighted by trichrome staining (trichrome staining, original magnification ×100)

The distribution of blood vessels was nonuniform: extravasated red blood cells, macrophages and other inflammatory elements were not present in the fibrous matrix. The blood vessels were lined by endothelium without evidence of pericytes. Endothelial cells were highlighted by CD34 immunostaining and did not show any significant alteration (Fig. 5). There was no evidence of inflammatory cells or thrombosis inside vascular lumina.

Discussion

Photodynamic therapy is a relatively new treatment modality based on the use of light-sensitive drugs called photosensitizers; the activation of the photosensitizer by

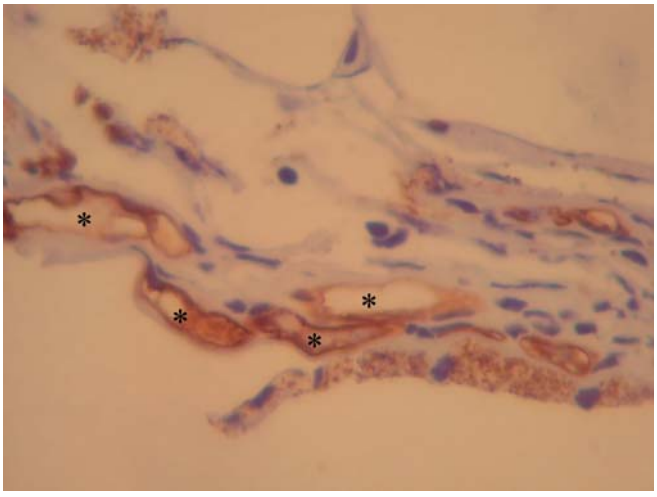

Fig. 5 Part of the membrane after PDT containing capillaries (*asterisks*) lined by endothelium without pericytes. The vessels shown have an irregular contour with no significant alteration of endothelial cells. There is no evidence of inflammatory cells or thrombosis inside vascular lumina (CD34 immunostaining, original magnification ×100)

laser light of appropriate wavelength initiates multiple photochemical reactions culminating in vessel occlusion of the target tissue.

Short-term efficacy of PDT in experimental models of CNV was assumed from the absence of fluorescein leakage from treated choroidal neovascular complex and confirmed by light microscopy, which showed occlusion of blood vessels by packed erythrocytes and fibrin [10, 11]. Husain et al. described long-term effects of PDT using verteporfin on experimental CNV in the cynomolgus monkey eye [11]; CNV lesions were still closed 4 weeks after treatment in 72% of eyes, light and electron microscopy of the occluded CNV demonstrating proliferating RPE envelopment of the neovascular membrane.

However, in animal models, CNV tends to involve spontaneously, as evidenced by RPE cells' envelopment and progressive decrease of angiographic leakage; this could affect model prediction of long-term effects of PDT [11, 13].

In humans, the safety and efficacy of PDT with verteporfin were evaluated in multicentre randomized clinical trials [14, 23, 24, 27]. These studies showed that a single PDT treatment could lead to occlusion of CNV vessels, as evidenced by cessation of fluorescein leakage from CNV, without adverse events. Since fluorescein leakage recurred in almost all cases between 1 and 3 months later, multiple PDT applications were performed [14, 19].

The Verteporfin in Photodynamic Therapy (VIP) Study Group evaluated the efficacy of PDT in pathologic myopia and in other non-AMD-related CNV [21]. In this study, fluorescein angiography revealed in all verteporfin-treated patients a significant reduction of lesion size and in nearly half of the patients a complete cessation of angiographic leakage 1 week after treatment.

The 1-year results of the VIP Study confirmed that the verteporfin-treated group was more likely to have smaller dimensions of CNV and greater chance of leakage absence compared with control group [27]. Sickenberg supposed that these findings might be due to the high regenerative potential of a healthy RPE [21]: photochemical occlusion of the neovascular lesion could allow surrounding RPE cells to migrate and proliferate, engulfing the CNV. Consequently, any further growth of the membrane is avoided.

Although the safety and the efficacy of PDT have been assessed in clinical trials [14, 19, 23, 27, 28], to date there have been few microscopic studies of PDT-treated CNV in humans.

Ghazi et al. were the first to describe the histopathologic and ultrastructural characteristics of a human AMD-related CNV excised 4 weeks after PDT. They observed clinicopathologic features similar to those of non-treated choroidal neovascular membranes, except for endothelial degeneration and vascular thrombosis in the peripheral vessels. Nevertheless, they reported the presence of some normal vessels with uninjured endothelial cells secondary to vascular regrowth or recanalization of previously occluded vessels that were thought to be responsible for the leakage seen on FA [4].

Schnurrbusch and coworkers reported the histological findings in two cases of predominantly classic AMD-related CNV removed surgically 14 weeks and 4 months after PDT. The specimens showed many vessels occluded by thrombotic masses with endothelial damage, whereas other vascular channels within the CNV were normal. RPE cell degeneration was noted in both specimens, and a possible correlation with PDT treatment was suggested [20]. Grossniklaus and associates described the clinicopathologic findings in a post-mortem eye from a patient with AMD 8 months after macular translocation surgery and 2 weeks after PDT for recurrent CNV. They found complete occlusion of the CNV vessels with platelet-fibrin thrombi, whereas the retinal and choroidal vessels were normally perfused [8].

Recently Moshfeghi et al. reported the histopathologic findings of eight choroidal neovascular membranes due to different diseases and removed after PDT [15]. All membranes were excised because of lack of response to PDT. Histological examination disclosed fibrovascular tissue containing inflammatory cells and intact vascular channels. Vascular damage, evidenced by extravasated erythrocytes, was noted in some membranes, whereas vascular occlusion was observed in one CNV excised 3 days after PDT treatment.

The membrane we describe was composed of fibrovascular tissue and showed features very similar to those

of previously reported excised myopic CNV without PDT [7]. The histopathologic findings in our patient demonstrated a small and remarkably thin CNV with a significant avascular component.

We did not observe any vascular occlusion, and blood vessels were considered unremarkable on the basis of light microscopy. The lack of occluded vessels within the CNV represents a considerable difference from the first histopathologic reports of PDT-treated CNV in humans [4, 8], but it is consistent with the recent report by Moshfeghi [15]. Furthermore, in our specimen no extravasated red blood cells or inflammatory elements were evident. Unlike previous clinicopathologic studies, our case showed a successful response to verteporfin PDT as demonstrated by visual acuity improvement and absence of fluorescein leakage from CNV at 1-month follow-up, probably as a result of blood vessel closure (Fig. 1B). The leakage of the dye 4 months after PDT suggested reopening or regrowth of new vessels as confirmed by histological examination. We hypothesize that the presence of normally perfused blood vessels without histologic degenerative changes in the endothelial cells can be explained by the long interval between PDT application and surgical excision of the membrane. Our specimen was removed 4 months after PDT; vascular occlusion may have resolved by this time and damage to endothelial cells may have been repaired. During this interval injured endothelial cells could have regenerated and clot fragmentation could have led to recanalization of pre-existing new vessels [5, 17].

A further possible explanation is regrowth of new vessels induced by the underlying pathology. A new angiogenic stimulation in response to transitory choroidal ischemia within the PDT-treated region [18] seems unlikely because of the long time between PDT and the recurrence of CNV.

Our findings are consistent with previous clinical trials and histologic studies demonstrating that PDT-induced occlusion is temporary and can be observed for short periods after treatment. However, it is not yet clear whether the presence of patent vessels in PDT-treated CNV is a consequence of regeneration or reperfusion of preexisting new vessels, and both mechanisms may be coexistent. In conclusion, verteporfin therapy seems unable to achieve a permanent occlusion of new vessels and fluorescein leakage from CNV can be considered a sign of blood vessel perfusion showing the need for retreatments.

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