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Choroidal neovascular membranes after photodynamic therapy: Ultrastructural analysis of two surgically excised membranes

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PURPOSE. *To report on the ultrastructural electron microscopic findings of two surgically excised subfoveal choroidal neovascular membranes (CNV) that had undergone photodynamic therapy (PDT).*
METHODS. *Two patients underwent PDT because of subfoveal neovascular membranes (CNV). Due to enlargement of the CNV seen on fluorescein angiography three months after PDT, one patient underwent surgical excision of the membrane; the other patient underwent both surgical membrane excision combined with macular translocation one month after PDT. The membranes were examined under the transmission electron microscope (TEM).*
RESULTS. *The membranes were composed of a core and a rim, the latter being mainly composed of fibrin and collagen fibrils. The core was preeminently composed of endothelium-lined vascular channels associated with retinal epithelium cells. The endothelial cells of blood vessels appeared well-preserved.*
CONCLUSIONS.*The lack of histological signs of recanalization and vascular thrombosis may indicate that in our cases the enlargement of the CNVs seen on fluorescein angiography three months and one month respectively after PDT may originate mainly from reproliferation of choroidal vessels rather than recanalization of previously occluded vessels. (Eur J Ophthalmol 2004; 14: 555-61)*

KEY WORDS. *Photodynamic therapy, Subfoveal choroidal neovascularization, Ultrastructural findings*

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INTRODUCTION

Age-related macular degeneration is the main cause of legal blindness in patients older than 55 years (1). It may lead to the formation of a choroidal neovascular membrane (CNV) in the subfoveal area, which is up to now difficult to treat. Laser photocoagulation of subfoveal CNV results in an immediate decline of vision (2). Coscas and coworkers (3) proposed perifoveal laser photocoagulation for the treatment of subfoveal neovascularization in patients with ARMD. Membrane excision has been performed with varying functional results (4). Recently, photodynamic therapy (PDT), which has been successfully applied in the treatment of some cancers (5), has found application in the treatment of subfoveal CNVs. It is based on the use of a light-sensitive agent, which when stimulated by light of a specific wavelength, leads to peroxidative reactions in cell membranes and cytoplasmic organelles, causing cell damage and cell death in the target tis-

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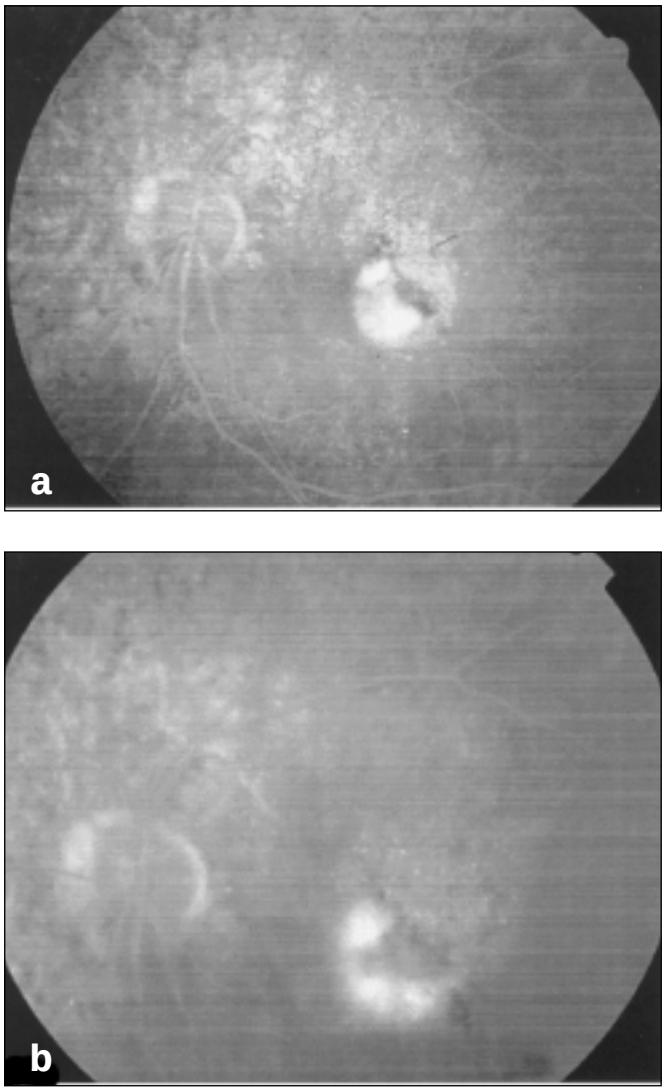


Fig. 1 - *Late fluorescein angiogram before* **a)** *and 3 months after photodynamic therapy PDT* **b)** *showing modified leakage with progression of the choroidal neovascular membrane.*

sue. In the case of a CNV, PDT leads to a photothrombosis in the neovascular network. In ophthalmology, PDT with verteporfin has become a commonly used treatment of subfoveal CNVs. Verteporfin has a high affinity for plasma proteins, especially low density lipoproteins (LDL) (6). The endothelium of neovascular tissue exhibits increased LDL receptors compared to the surrounding neovascular endothelium. The activation of the photosensitizer causes endothelial cell damage, thrombus formation, and vascular occlusion (7).

However, PDT is associated with a high rate of recurrence and their effects have been shown to be usually temporary by clinical experience (8). It is not clear

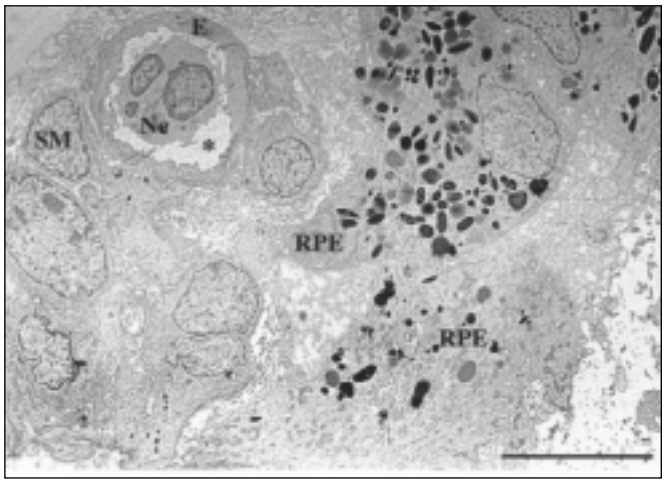


Fig. 2 - *Portion of the core of the neovascular membrane. On the left a vascular channel (*) with a neutrophil (Ne) inside lined by endothelial cells (E). On the right some retinal pigment epithelial cells (RPE) characterized by the presence of numerous electron-dense, lancet-shaped, melanin granules in the cytoplasm. SM = Smooth muscle cell. Scale bar = 10 µm.*

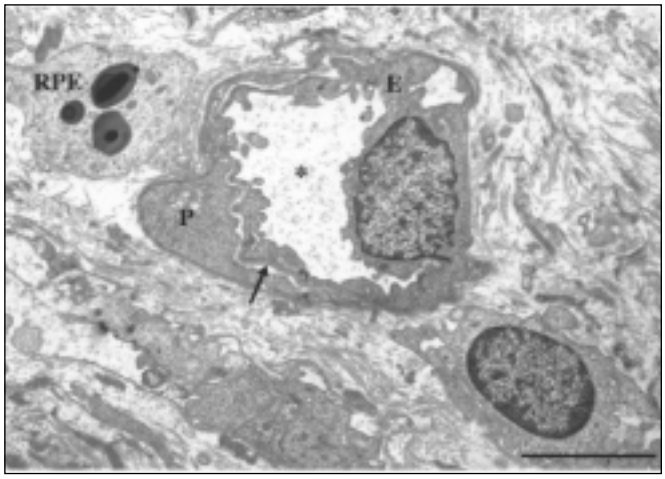


Fig. 3 - *A vascular channel (*) lined by the endothelium (E) and completely surrounded by a pericyte (P). Arrow indicates the endothelium basement membrane. RPE = Retinal pigment epithelium. Scale bar = 2.5 µm.*

whether recurrences are caused by reperfusion of previously occluded vessels or whether they originate from reproliferation of choroidal vessels.

PATIENTS AND METHODS

Case 1

A 72-year-old highly myopic pseudophakic woman with a subfoveal CNV had previously undergone three

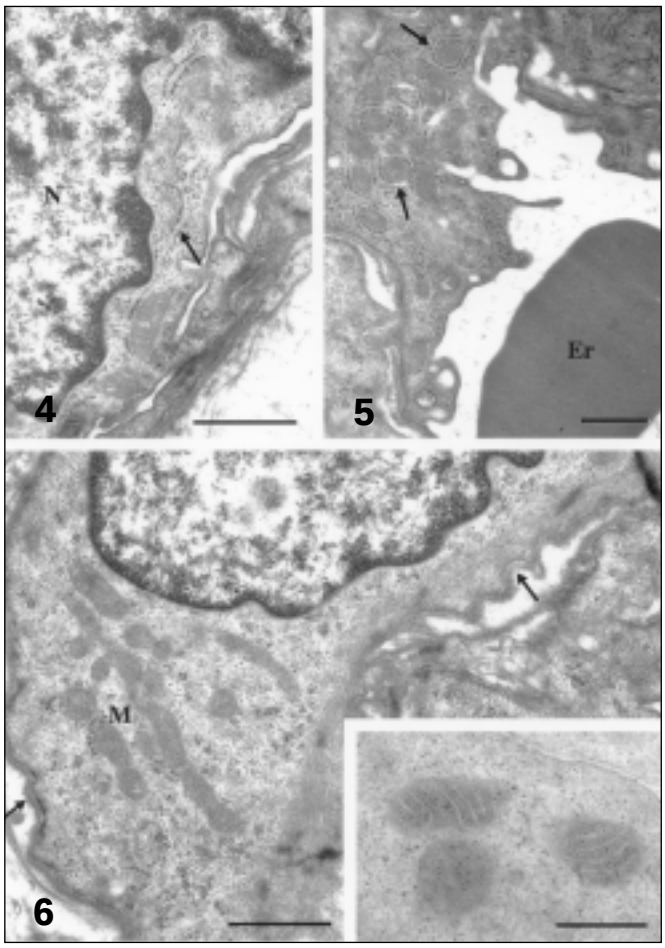


Fig. 4 - *Endothelial cell showing portion of the nucleus (N) with clumps of chromatin adjacent to the inner nuclear membrane. Numerous free ribosomes, and short RER cisternae (arrow) are visible in the cytoplasm. Scale bar = 1 µm.*

Fig. 5 - *Endothelial cell with short RER cisternae (arrow) surrounding mitochondria. Er, = Erythrocyte. Scale bar = 1 µm.*

Fig. 6 - *Endothelial cell. Numerous free ribosomes and elongated, intact mitochondria (M) are present in the cytoplasm. Arrows point to the basement membrane. Scale bar = 1 µm. In the inset well-structured mitochondria with an electron-dense matrix and intact parallel cristae. Scale bar = 0.5 µm.*

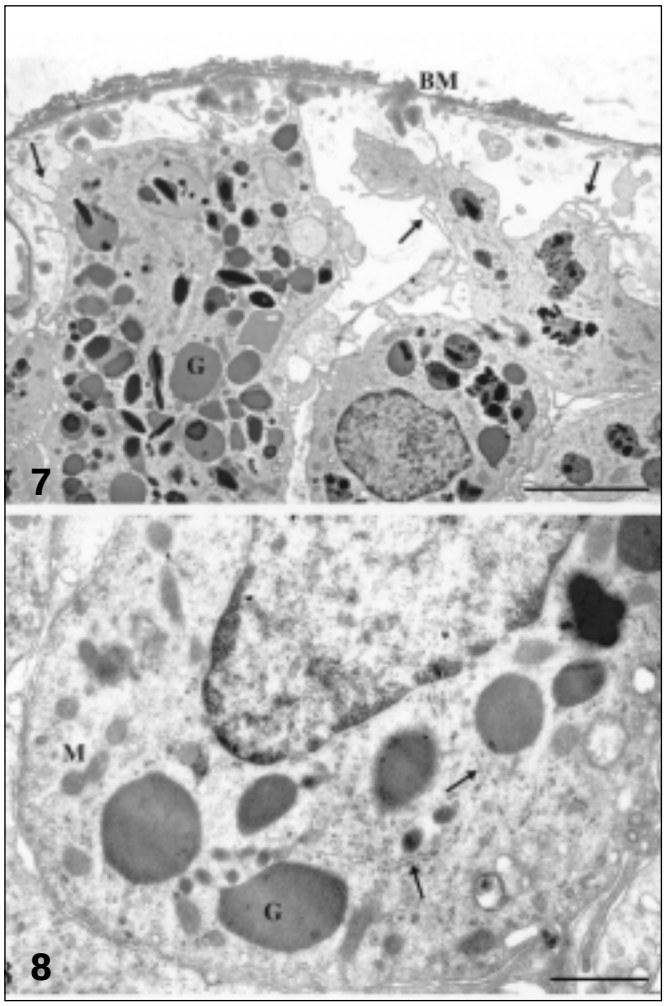


Fig. 7 - *Normal retinal pigment epithelial RPE cells with apical microvilli (arrow). Irregularly- shaped granules (G) of different electron density are present in the cytoplasm. BM = Bruch's membrane. Scale bar = 5 µm.*

Fig. 8 - *Portion of a retinal pigment epithelial RPE cell with a nucleus containing some peripheral clumps of chromatin. RER cisternae (arrow), mitochondria (M), and electron-dense granules (G) are visible in the cytoplasm. MV, = Microvilli. Scale bar = 1 µm.*

series of PDT according to the guidelines of the TAP study (9). Three months after the last application of PDT a recurrence of the CNV was evident on fluorescein angiography. Best-corrected visual acuity had declined to 0.02. Membrane excision was proposed to the patient as an alternative therapy. We performed a pars plana vitrectomy, the membrane was excised via a small retinotomy, and the globe filled with balanced salt solution. The neovascular membrane was immediately fixed for electron microscopic studies.

Case 2

An 80-year-old woman had a subfoveal classic CNV (Fig. 1a) and a best-corrected visual acuity of 0.2 with -1.0 diopters. The patient underwent only one session of PDT because she suffered from an intense back pain during the verteporfin infusion (only 17 ml of the drug was administrated). According to the TAP study this should be enough to achieve a photodynamic effect. The pain was so strong that the infusion had to be stopped

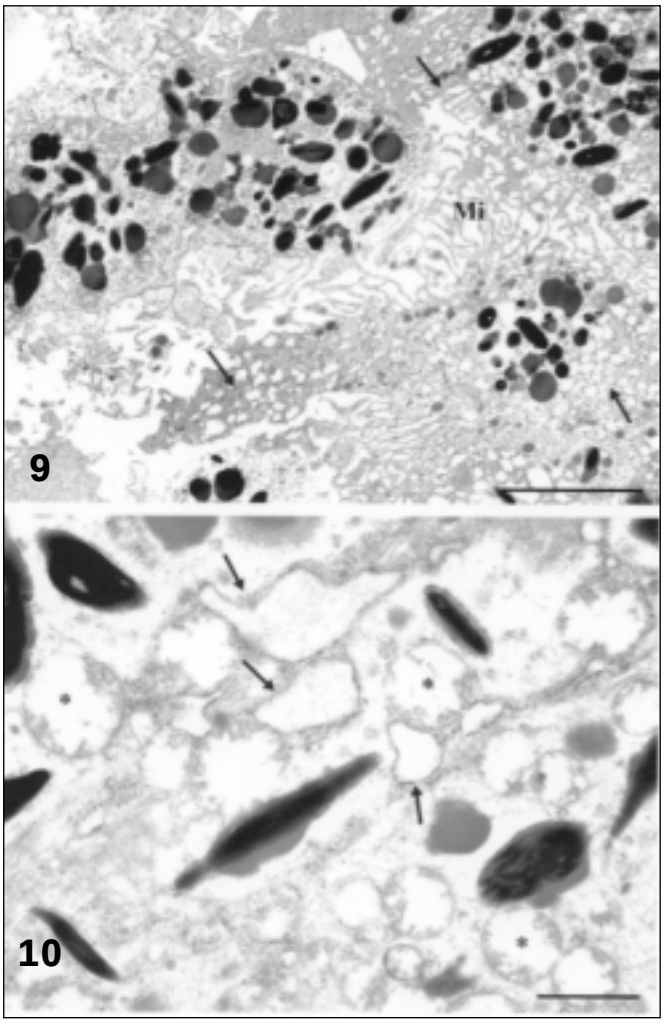
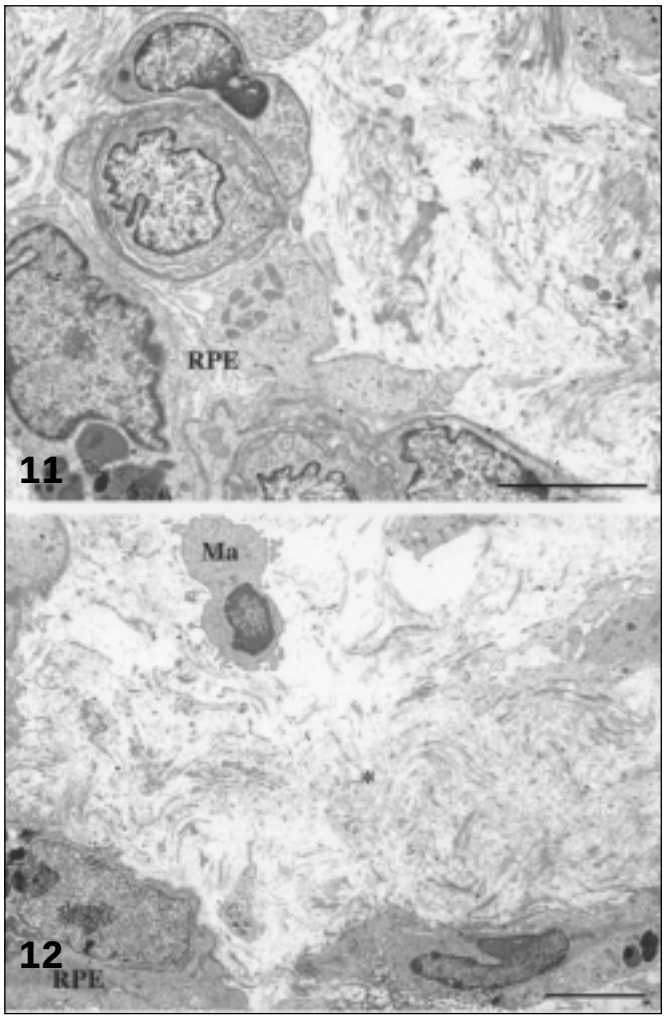


Fig. 9 - *Portion of several altered retinal pigment epithelial cells showing a vacuolized cytoplasm (arrow). Mi = Microvilli. Scale bar = 5 µm.*
Fig. 10 - *Altered retinal pigment epithelial RPE cell. Hypertrophic, damaged mitochondria (*) and swollen cisternae of RER (arrow) are visible in the cytoplasm. Scale bar = 1µm.*



Figs. 11-12 - *Portion of the rim of the neovascular membrane. Fibrin and collagen fibrils appear loosely arranged (*). Ma = Macrophage; RPE = Retinal pigment epithelium. Scale bars = 5 µm.*

and the patient could not support another treatment. One month after the treatment the fluorescein angiography showed a modified leakage with progression of the CNV (Fig. 1b). Therefore, and because of the intense back pain the patient had during verteporfin infusion, we proposed surgical treatment. We performed a macular translocation with a 360° retinotomy and membrane excision. The best-corrected visual acuity reached the value of 0.1 six months after surgery.

Ultrastructural methods

Neovascular membranes were promptly fixed in 3% glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.2) for 1 hour at 4 °C and postfixed with 1% osmium tetroxide in the same buffer for 2 hours at room temperature.

After dehydration in an ascendant series of ethanols, the specimens were embedded in Epon-araldite resin.

Serial ultrathin sections (50-80 nm) were cut with a Reichert-Jung Ultracut E equipped with a diamond knife, placed on formvar-carbon coated grids, stained with an aqueous solution of uranyl acetate and lead citrate, and finally examined with a Jeol 100 SX transmission electron microscope (TEM).

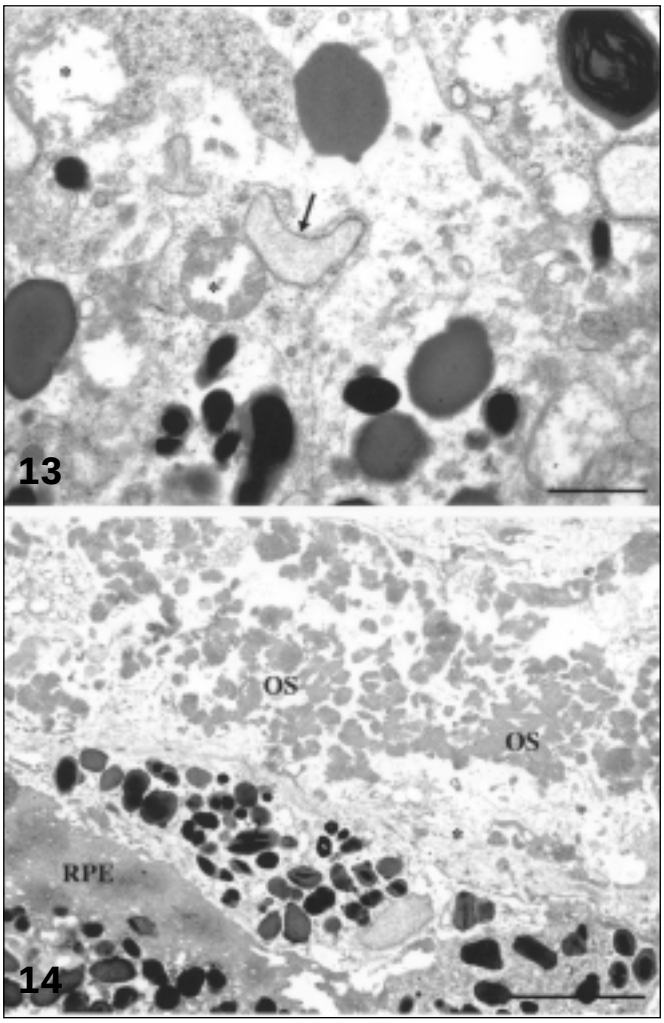


Fig. 13 - *Portion of the damaged cytoplasm of a retinal pigment epithelial cell. Hypertrophic mitochondria (*) showing cristolysis, and dilated RER cisternae (arrow) are visible. Scale bar =1 µm.*
Fig. 14 - *External portion of the neovascular membrane. Scattered outer photoreceptor segments (OS) intermingled with fibrin (*) are present. RPE = Retinal pigment epithelium. Scale bar = 5 µm.*

RESULTS

Electron microscopy:

The examined subfoveal CNVs were composed of two regions, a core and a rim, as previously described by Lopez et al (10).

Case 1

In the first case the core was mainly composed of endothelium-lined vascular channels associated with the retinal pigment epithelium (RPE) (Fig. 2). The vessel endothelium appeared well preserved. The roundish nuclei of the endothelial cells had a normal aspect with scattered clumps of chromatin mainly adjacent to the inner nuclear envelope (Figs. 3, 4). In the cytoplasm numerous free ribosomes, some short cisternae of rough endoplasmic reticulum (Figs. 4, 5), and intact mitochondria with a dense matrix and well-arranged cristae were visibile (Figs. 5, 6). Neither discontinuity nor disintegration of the endothelial cell layer was observed. The endothelial basal lamina appeared continuous (Fig. 6). Endothelial cells were often surrounded by pericytes (Fig. 3). In the same specimen the majority of RPE retinal pigment epithelium cells were well preserved (Fig. 7). The nucleus contained mainly diffuse chromatin and in the cytoplasm numerous free ribosomes, cisternae of rough endoplasmic reticulum, and scattered, irregularly- shaped electron-dense pigment granules were visible (Figs. 7, 8). Mitochondria appeared well structured. The cell surface showed numerous microvilli (Fig. 7). By contrast, some areas of the RPE retinal pigment epithelium showed signs of degeneration. In these areas the cell cytoplasm was less dense and appeared vacuolized (Figs. 9, 10). Enlarged cisternae of rough endoplasmic reticulum and grossly damaged mitochondria showing an increased volume (hypertrophy), cristolysis, and matrix dilution were visible (Fig. 10). Electron-dense pigment granules appeared to maintain a normal structure. The rim surrounding the core was mainly composed of fibrin and collagen fibrils (Figs. 11, 12). Occasionally some macrophages were visible. No photoreceptor outer segments were detected in this area.

Case 2

In the second case the micrographs showed ultrastructural features similar to those of Case 1. The vessels within the core, although much less numerous, showed a well-preserved endothelium. The RPE was more damaged, showing vacuolization of the cytoplasm and hypertrophic mitochondria (Fig. 13). The rim was composed of collagen fibrils with numerous intermingled photoreceptor outer segments (Fig. 14).

DISCUSSION

Histological and ultrastructural findings from CNVs treated with PDT are relatively rare and difficult to ob-

tain. Even if the two cases described by us are different from each other regarding the clinical history, PDT performance, and time between last PDT and excision, the histological results are similar: we found well-preserved vascular endothelium cells and no occluded vessels in both cases.

Schnurrbusch et al (11) examined the surgically excised membranes of two patients affected with initially predominant classic CNV 4 months after PDT. Both membranes were enlarged 3 months after PDT.

They found occluded vessels containing thrombotic material as well as perfused vessels containing normal blood cells and observed ultrastructural damage of the endothelial cells, which was probably induced by PDT. The authors concluded that the recurrence of the CNV 3 months after PDT was related to new vessel growth and recanalization of previously occluded vessels.

Schmidt-Erfurth and coworkers (12) found that recanalization is illustrated histologically by reformation of novel lumina within previously occluded channels. Electron microscopy in experimental models showed duplication of vascular basement membranes indicative of recanalization.

We did not observe any of these findings in our specimens. Therefore we could assume that in our cases reproliferation can explain the recurrence of the CNV, or that the thrombotic effects caused by PDT had disappeared after 3 months, even if Schnurrbusch found occluded vessels after 4 months.

In a recent paper article, Schmidt-Erfurth and coworkers found that PDT may stimulate VEGF expression as well as VEGFR-3 and PEDF upregulation. VEGF is a stimulator for neovascularization and its expression is upregulated by hypoxia. Continuous recurrence during multiple PDT courses is probably the consequence of VEGF stimulation reinforced by the PDT itself (13). This could explain in part the recurrence of CNV in our cases.

In the second case PDT may not have obtained the necessary effect since only 17 ml of verteporfin was administrated. Even though according to the TAP study recommendations this should have been enough to cause some effect, we suppose that it was not sufficient. It could be that the PDT had little effect on the target tissue, leaving a great part of the neovascular blood vessels undamaged.

In both cases some areas of the RPE showed signs of degeneration. These findings have also been described by Schnurrbusch and coworkers (11), who found significant degeneration of RPE cells. There is no evidence that this was an effect due to PDT, since RPE cell atrophy can also be caused by the age-related macular degeneration itself, but a direct effect of PDT cannot be excluded since RPE cells express LDL receptors (14, 15).

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