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TRANSPUPILLARY THERMOTHERAPY AND PHOTODYNAMIC THERAPY FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION

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To Philippa and Nicoline

ABSTRACT

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness among elderly in industrialized nations, and promises to extract an even greater toll with the imminent demographic shift. Neovascular AMD (wet AMD) often develops quickly and involves the growth of new blood vessels under the retina (choroidal neovascularization, CNV). These new blood vessels tend to be fragile and often leak blood and fluid. The blood and fluid elevates the macula, the central part of the retina, causing rapid visual loss. Without treatment the prognosis is poor with profound impact on an individual's ability to perform daily tasks.

Photodynamic therapy (PDT) has been the most common treatment for neovascular AMD. PDT uses a cold laser to seal the leaking blood vessels. This involves injecting a lightsensitive drug that reaches and coats the abnormal blood vessels via the blood stream. The drug is then activated by light leading to a local occlusion of new vessels.

Transpupillary thermotherapy (TTT) is technique in which a laser-induced subretinal vascular occlusion can be created through a small temperature elevation but without any photosensitive drugHowever, there has been a controversy about the optimal TTT laser intensity and controlled clinical trails demonstrating efficacy in neovascular AMD have been lacking.

The first two studies of the thesis demonstrate that PDT as well as TTT can reduce experimental CNV, without causing damage to the surrounding tissue. A cellular damage in surroundning tissues was however seen at higher dosage. The therapeutic window is thus narrow for both treatments underscoring the importance minimizing treatment doses. We also found that both TTT and PDT induce an immediate thrombosis and cessation of perfusion in CNV areas, but after PDT some areas remained vascularised while after TTT the closure of the abnormal vessels proceeded for at least one week.
The third and fourth studies of the thesis were on a prospective clinical study, randomizing 98 patients with neovascular AMD (occult CNV) to either low-dose TTT or PDT. During a follow-up of 12 months, no significant differences between the two groups emerged. The proportion of patients with stabilized visual acuity was approximately 75% in both groups and the two treatments were equally potent at stabilizing patient-reported visual function.

Recently intravitreal anti-VEGF has become the first line treatment for neovascular AMD demonstrating superior efficacy for all forms of neovascular AMD. However, anti-VEGF is expensive and requires repeated injections. The use of PDT as an adjuvant to anti-VEGF therapy has been suggested to decrease both the cost and the need for repeated injections. Also this combination may prove beneficial regarding control of lesion growth. The results of this thesis, showing that low dose TTT may be equipotent to PDT, suggests that TTT may be a cost-effective adjuvant to intravitreal anti-VEGF treatment.

LIST OF PUBLICATIONS

I. Yue Ming, Peep Algvere, Anne Odergren, Lennart Berglin, Ingeborg van der Ploeg, Stefan Seregard, Anders Kvanta. Subthreshold transpupillary thermotherapy reduces experimental choroidal neovascularization in the mouse without collateral damage to the neural retina.
Invest Ophthalmol Vis Sci 2004;45:1969-1974

II. Anne Odergren, Yue Ming, Anders Kvanta. Photodynamic therapy of experimental choroidal neovascularization in the mouse.
Curr Eye Res 2006;31:765-774

III. Anne Odergren, Peep Algvere, Stefan Seregard, Anders Kvanta. A prospective randomized study on low dose transpupillary thermotherapy (TTT) versus photodynamic therapy (PDT) for neovascular age-related macular degeneration. Br J Ophthalmol 2008;92:757-761

IV. Anne Odergren, Peep Algvere, Stefan Seregard, Carina Libert, Anders Kvanta. Vision-related function after low dose transpupillary thermotherapy (TTT) versus photodynamic therapy (PDT) for neovascular age-related macular degeneration. (submitted)

CONTENTS

LIST OF ABBREVIATIONS

AMD	Age-related macular degeneration
BCVA	Best corrected visual acuity
CNV	Choroidal neovascularization
ECM	Extracellular matrix
ETDRS	Early treatment diabetic retinopathy study
GLD	Greatest linear diameter
MPS	Macular photocoagulation study
NEI VFQ 25	National Eye Institute visual function questionnaire
PDT	Photodynamic therapy
RPE	Retinal pigment epithelium
TTT	Transpupillary thermotherapy
VEGF	Vascular endothelial growth factor

1 INTRODUCTION

1.1 THE RETINA AND ITS FUNDAMENTALS

The retina is a multi-layered sensory tissue that lines the innermost back of the eye. It is made up of cells with highly specialized functions. Alongside the outer monolayer of the retinal pigment epithelium (RPE), is the inner neurosensory retina consisting of photoreceptor cells, bipolar cells, ganglion cells, horizontal cells, amacrine cells and interplexiform cells as well as glia elements.

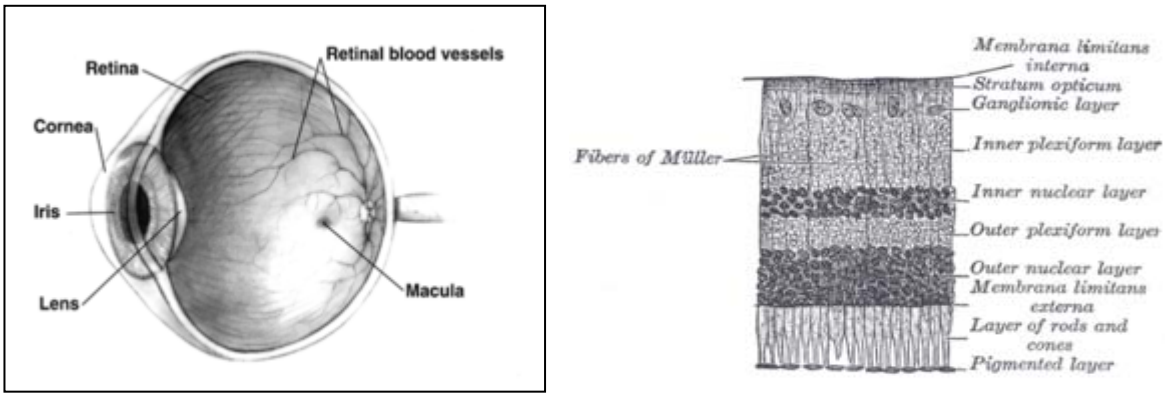

Figure 1. The retina is composed of ten distinct layers, starting with photoreceptors cells, and moving inward to the layer that includes modulating interneurons, and synapsing finally on optic nerve cells.

The photoreceptor cells capture light rays and convert them into electrical impulses. These impulses travel along the optic nerve to the brain where they are turned into images. There are two types of photoreceptors in the retina: rods and cones and the retina contains approximately 6 million cones and 125 million rods. The cones are mainly concentrated to the macula, the portion of the retina responsible for central vision. The cones are densely packed within the fovea (Fig 2), the very central portion of the macula that mediates a vision marked by high temporal and spatial resolution and color (Curcio et al 1990). The rods are spread throughout the peripheral retina, subserving a peripheral vision with comparatively low resolution and high sensitivity but lacking in color information (Curcio et al 1990).

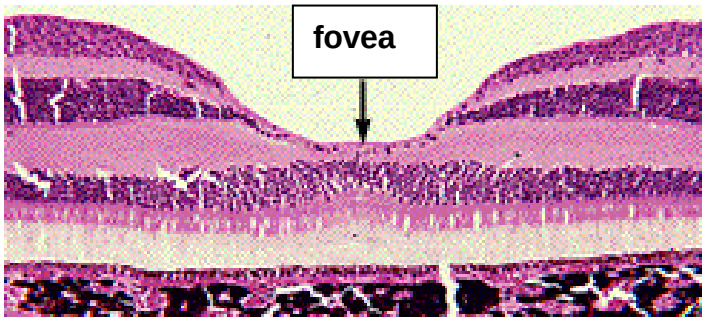

Figure 2. The fovea is a thin zone of retina composed exclusively of cones. One cone synapses with one bipolar cell which links to one ganglion cell. These anatomic features enhance resolution och make the fovea the center of vision.

1.2 AGE-RELATED MACULAR DEGENERATION (AMD) AND DEFINITIONS

AMD can be subgrouped into three forms, one early and two advanced (geographic atrophy and neovascular (also termed exsudative or wet) AMD (AREDS report nr 3, 2000). Geographic atrophy is characterized by a progressive, and often slow, RPE loss in the macula.

Neovascular AMD that accounts for approximately 80 percent of cases with severe vision loss (Miller et al 1995, Schnurrbusch et al 2001, Ferris et al 1984) is characterized by a subretinal ingrowth of vessels originating from the choroid (i.e. choroidal neovascularization, CNV) (Ambati et al 2003, Green et al 1977, Kwak et al 2000, Rakic et al 2003). The new vessels are fragile and a serous leakage of blood and proteins is often seen. The end stage is a scar formation. The progress of the disease can be rapid, with acute loss of vision and contrast sensitivity (VIP report 2, 2001, TAP report 2 2001, TAP and VIP report No1 2003).

With time, neovascular AMD usually affects both eyes, (MPS study group 1993 and 1997, Pieramici&Bressler 1998, Bressler et al 1990, Chang et al 1995, Wang et al 1998, Bartlett et al 2003). Without treatment the prognosis is poor, and in cases affecting both eyes the impact on an individual´s ability to perform daily tasks, such as reading, writing, driving and social functioning, is profound, leading to progressive loss of independence (Brown et al 2002, Cahill et al 2005, Stein et al 2003, Slakter et al 2005, AREDS report No 10 2003).

1.3 PREVALENCE AND INCIDENCE OF AMD

Neovascular AMD is a primary cause of blindness in the Western world (Augood et al 2006, Vingerling et al 1995, Mitchell et al 1995, Klein et al 2002). The disease affects around two million people over the age of 70 years, in Europe.
The prevalence for AMD increases with age and the disease rarely occurs in people under the age of 60 years (Klein et al 2002, Smith et al 2001, Haddad et al 2006).

At the age of 60-65 years the risk of acquiring AMD is around 5 percent increasing to nearly 30 percent in individuals over the age of 75 years.
In Sweden it is estimated that around 300,000 citizens suffer from some kind of AMD and that 45,000 has a severe vision loss due to neovascular AMD

About 40 percent of people with wet AMD in one eye progress to wet AMD in the fellow eye within five years (Solomon et al 2007, AREDS report No 19 2005)

1.4 RISK FACTORS

The greatest risk factor is age. Other risk factors include:

1. family history (those with immediate family members who have AMD are at a higher risk of developing the disease highlighting the important role of genetic factors),
2. smoking,
3. race (Caucasians are more likely to lose vision from AMD than African Americans),
4. gender (women appear to be at greater risk than men).

1.5 CLASSIFICATIONS OF NEOVASCULAR AMD

Neovascular AMD is classified into predominantly classic, occult and minimally classic (VIP, TAP study group 2002).

This classification is based on the appearance of the new blood vessel growth in the macula as imaged by fluorescein angiography (Fig 3) (MPS group 1991). There are further sub-classifications based on factors such as the specific location of CNV in relation to the fovea (The international ARM epidemiological Study Group 1995).

AMD classification and subclassification is important because it decides the type of treatment used.

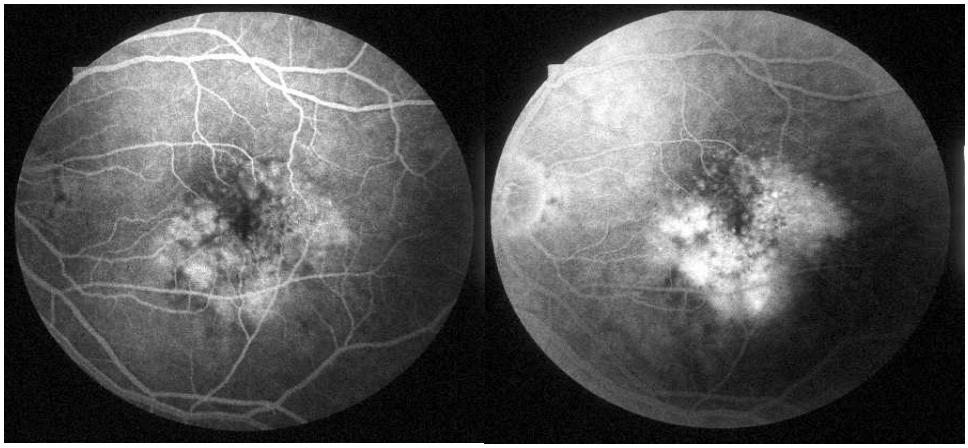

Figure 3. Retinal photography and intravenous fluorescein angiography. CNV characteristically leaks fluorescein and intravenous fluorescein angiography. Left and right: early and late frames. The late and diffuse leakage on the fluorescein angiogram shown here is typical of occult CNV.

1.5.1 Predominantly Classic

This subtype refers to a well-defined area of new blood vessel growth in the macula (MPS group 1991, Freund et al 1993). Predominantly classic AMD has the most aggressive disease pathology and accounts for approximately 25 percent of neovascular AMD cases. Studies suggest that this subtype leads to more rapid vision loss than the other subtypes. (MPS study group 1991, TAP Report No 1 1999, Ali et al 2004, Hogg et al 2003, Fernandes et al 2002, Margherio et al 2000)

1.5.2 Occult

In the occult subtype the CNV lesion is not well defined, and the leakage is less pronounced than in the predominantly classic subtype. The vision loss also progresses slower than in the predominantly classic subtype. Together with the minimally classic CNV, occult CNV accounts for 75 percent of wet AMD cases (VIP Report No 2 2001, Pauleikhoff 2005, Polito et al 2006)

1.5.3 Minimally Classic

In this subtype the well-defined area of new blood vessel growth accounts for less than 50 percent of the total CNV area.

1.6 SYMPTOMS

The typical early symptom of neovascular AMD is metamorphopsia, i.e.straight lines appear crooked. A blurred central vision may also appear in wet AMD. This results from the leaking blood vessels causing submacular fluid with distorted vision. As the disease progresses, neovascular AMD also tends to produce central scotomas as a result of blood or fluid under the macula. (Fig 4)

1.7 PATHOGENESIS

The cause of CNV is multifactorial, involving oxidative and inflammatory components as well as angiogenesis (Ambati et al 2004). Some histopathological changes in the macula such as the increased amount of connective tissue and metabolic waste products in Bruch´s membrane, and vascular changes of the choroid can be attributed to normal ageing, (Sarks 1976).Certain histological and cellular events including subretinal deposits (basal laminar deposit and membranous debris) and macrophage infiltration are on the other hand suggested hallmarks of progression into AMD (Sarks et al 2007). Nevertheless, much knowledge on early disease progession is lacking as is the reason for the later progression into the two distinct forms, geographic atrophy and neovascular AMD.

1.8 ANGIOGENESIS

Angiogenesis, the formation of new vessels from pre-existing ones, is a major cause of severe vision loss. In neovascular AMD choroidal vessels grow through Bruch´s membrane into the subretinal space, causing subretinal exudation and haemorrhage (Ambati el al 2003).

Angiogenesis is a multistep process that involves the stimulation of angiogenic growth factor receptors on vascular endothelial cells, proteolytic breakdown of the endothelial cell basal membrane, endothelial cell proliferation and migration, degradation of the surrounding extracellular matrix (ECM), vessel maturation,

recruitment of supporting cells (e.g. pericytes) and finally closure of the newly formed arteriovenous loops (Folkman 1971,Yancopoulus et al 2000, Carmeliet 2003).

Each of these steps is tightly regulated by the action of both stimulatory (angiogenic factors) and inhibitory (angiogenic inhibitors) molecules (Carmeliet & Jain 2000).

In the normal state, the vessels are quiescent as the action of the angiogenic inhibitors dominates. Under certain conditions, such as hypoxia or inflammation, that activate angiogenic factors, the balance may shift in favour of angiogenesis, an event termed the 'angiogenic switch' (Kvanta 2006).

Among the angiogenic growth factors the role of vascular endothelial growth factor (VEGF) has been particularily studied both in the eye and in other organs (Senger et al 1986; Leung et al 1989).

For example, retinal hypoxia leads to a pronounced increase in VEGF levels in several cell types, including retinal endothelial cells, pericytes, Müller cells and RPE cells (Adamis et al 1993; Aiello et al 1995).

Experimental retinal neovascularization is also associated with increased VEGF levels (Miller et al 1994; Pierce et al 1995) and inhibition of VEGF reduces neovascularization (Aiello et al. 1995; Robinson et al. 1996). Furthermore, VEGF has been found in CNV lesions in animal models of choroidal neovascularization (Ishibashi et al 1997, Yi et al 1997) and inhibition of VEGF (Kwak et al 2000; Reich et al 2003, Krzystolik et al 2002) reduces CNV formation.

Whether VEGF is sufficient to create CNV is still unclear as results are conflicting (Wang et al 2003; Cui et al 2000, Oschima et al 2004). The exact mechanism by which VEGF stimulates CNV formation also remains unclear. Most studies support a role for inflammation-mediated triggering of VEGF and the angiogenic response (Kvanta 1995; Zhou et al 2005), but it has been shown that hypoxic stimulation of VEGF may be important in CNV too (Vinores et al 2005).

Production of VEGF has been demonstrated in several cell types associated with CNV, including RPE cells, choroidal fibroblasts and infiltrating neutrophils (Adamis et al. 1993; Kvanta 1995, Zhou et al 2005) Clinical evidence for a role of VEGF in neovascular AMD comes from either post-mortem specimens (Kliffen et al 1997) or from analysis of surgically excised CNV membranes (Frank el al 1996; Kvanta et al 1996; Lopez et al 1996).

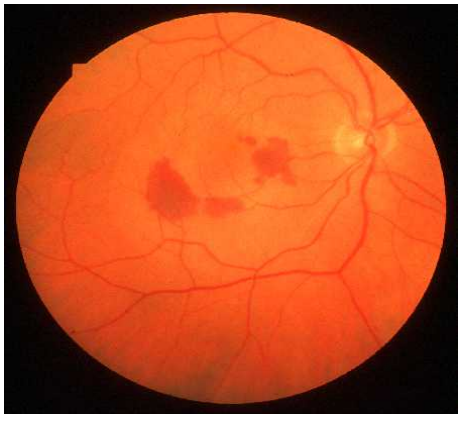

Figure 4. Neovascular AMD with macular haemorrhage

1.9 CNV THERAPY

Several treatments for neovascular AMD have emerged, all aimed at either halting or destroying CNV.

1.9.1 Laser photocoagulation

This procedure was the first clinically proven treatment for neovascular AMD (MPS study group 1990, MPS study group 1991, MPS study group 1994,Virgili&Bini 2007). It uses a laser to destroy the fragile, leaky blood vessels, preventing further loss of vision. Since the blood vessels are beneath the retina, the laser has to pass through the retina to reach them, resulting in damage to the retina as well. Laser is therefore only suitable for leaky blood vessels that have developed away from the central part of the macula.

1.9.2 Photodynamic therapy (PDT)

Numerous large-scale trials have shown that PDT with verteporfin (a photosensitising dye) and a low laser setting stabilizes visual acuity in patients with classic, predominantly classic and small occult subfoveal lesions secondary to AMD (TAP report 2, 2001, VIP report 2, 2001, TAP and VIP report No 1 2003, TAP report 3 2002).

The desired effect of PDT is to create a permanent vascular occlusion within the CNV lesion without causing damage to the overlying neural retina (Miller et al 1995). Clinical experience with PDT has demonstrated some limitations in treatment efficacy. For instance, many patients need more than one PDT treatment to stop CNV leakage, and, despite successful control of the leakage, most lesions will continue to grow. Vision already lost can not be restored by the treatment and therefore the majority of patients will experience a decrease in visual acuity, although at a lower rate than without treatment. This suggests that the treatment outcome of PDT is influenced by biological effects that may not be optimally controlled including potential injury to the surrounding neural retina and progression of angiogenesis.

1.9.3 Transpupillary thermotherapy (TTT)

In TTT, vascular occlusion is induced without the use of photosensitive dye, by delivering radiation at near infrared intensity (810 nm) to the target tissue through the

pupil (Rogers &Reichel 2001). A low increase in temperature (less than 10 C°) is applied to the CNV area.

Several case reports have indicated that TTT may be used to successfully treat CNV in patients with AMD, resulting in a high closure rate and resolution of the CNV complex without apparent retinal complications (Reichel et al 1999, Newsom et al 2001, Algvere et al 2001, Algvere et al 2003, Thach et al 2003). There are however no published randomized clinical trials demonstrating that TTT is efficient at controlling CNV. Also, concerns have been raised about the clinical safety of TTT for CNV since an optimal treatment effect seems to be achieved only at subthreshold doses, making under- and overtreatment a potential problem (Thompson et al 2001, Salinas-Alamán et al 2003).

1.9.4 Anti-vascular endothelial growth factor (VEGF)

Neovascular AMD can be managed by anti-angiogenesis drugs, aimed at decreasing blood vessel growth and leakage. Most of these act by blocking the VEGF protein. Anti-VEGF treatment of neovascular AMD has a favourable effect on disease progression and the vast majority of patients treated will have stabilized or improved vision (Rosenfeld et al 2006,).
However, for optimal effect, these agents require intravitreal injections for a considerable time, making this treatment a heavy burden for the the patient as well as for the healthcare system.

Furthermore, anti-VEGF treatment alone is not sufficient to induce regression of the neovascular lesions suggesting that angiogenic factors other than VEGF may be involved. It is likely that future treatments will address this and that we will have combinations of angiogenesis inhibitors that simultaneously target several angiogenic factors.

1.10 THE SOCIAL BURDEN OF AMD

Good vision if often taken for granted. It is important for a socially fulfilling and active life.

However, advanced AMD, particularly when affecting both eyes, seriously impairs any individual’s quality of life.

The loss of central vision will for instance make many ordinary tasks a challenge. Most people with AMD are past their retirement age and the disease´s effect on lost earnings and decreased productivity is thereby reduced.
Nevertheless, the cost for the society related to AMD, including the cost for treatment, caretaking services, falls and injuries as well as rehabilitation are considerable. In addition,

there is a cost for the individual for non-medical devices and medical aid. Furthermore, there are social and psychological consequences associated with visual impairment, such as depression, dependency and isolation.

2 AIMS OF THE STUDY

1. To analyze the effect of TTT on the normal mouse retina and in experimental CNV using different laser power settings that deliver subtreshold or threshold doses.
2. To analyze the effect of PDT on the normal mouse retina and in experimental CNV in order evaluate the qualitative and quantitative effects of this treatment in further detail.
3. To conduct a prospective and randomized clinical study comparing the effect of low dose TTT with PDT in occult CNV secondary to AMD.
4. To compare the effect of low dose transpupillary thermotherapy (TTT) and verteporfin photodynamic therapy (PDT) on patient-reported visual function using the National Eye Institute Visual Function Questionnarie 25 (NEI VFQ-25) in patients with occult neovascular AMD.

3 PATIENTS

Patients were recruited consecutively from the out patient retina clinic at S:t Eriks Eye Hospital and from the Department of Ophthalmology at Södersjukhuset, Stockholm .

The investigation adhered to the tenets of Helsinki and was approved by the Local Ethics Committee at the Karolinska Institutet.

All patients includes provided a signed informed consent form agreeing to participate.

4 MATERIAL AND METHODS

4.1 TTT FOR EXPERIMENTAL CNV IN THE MOUSE (PAPER 1)

4.1.1 TTT

TTT was delivered by an infrared diode laser emitting at 810 nm, and the treatment was performed for 60 seconds with a power setting of 50, 60, 70 or 80 mW to obtain thermal burns ranging from invisible (subthreshold) to visible (threshold). For each power setting, a series of four laser spots was delivered to the posterior pole of the mouse retina.

4.1.2 Induction of CNV

CNV was generated by a krypton laser-induced rupture of Bruch´s membrane. Only eyes in which a subretinal bubble (i.e. Bruch's membrane rupture) was formed for each burn were included in the study.

4.1.3 TTT of induced CNV

TTT was delivered as in 4.1.1.

4.1.4 Follow up

Eyes were enucleated 7 days after TTT and prepared for histological analysis. The CNV complex was evaluated on haematoxylin-eosin stained serial sections by measuring the maximum height of the CNV lesions. Ultrastructural changes of CNV membranes were examined by transmission electron microscopy.

4.2 PDT FOR EXPERIMENTAL CNV IN THE MOUSE (PAPER 2)

4.2.1 PDT

One minute after administration of verteporfin, given as a bolus dose into the tail vein, PDT was applied to the normal mouse retina or laser induced CNV lesions.

4.2.2 Induction of CNV

Induction of CNV was done with a krypton laser, as in 4.1.2.

4.2.3 PDT of induced CNV

The CNV lesions were treated using light doses of 32, 64 and 83 s.

4.2.4 Follow up

Eyes were enucleated 7 days after PDT. The enucleated eyes were processed for light and transmission electron microscopy. To evaluate the effect of PDT on CNV membranes, haematoxylin-eosin stained sections were examined. The maximal thickness of the CNV was estimated on histological sections and CNV area measured on choroidal flat mounts.

4.3 PDT VERSUS TTT FOR CNV - A PROSPECTIVE RANDOMIZED STUDY (PAPER 3 AND 4)

4.3.1 Study design

98 patients (98 eyes) were randomly assigned (in a 1:1 ratio) to receive either low dose TTT (136 mW/mm) (and sham PDT) (n= 52) or PDT (and sham TTT) (n=46), with re-treatment carried out if leakage was documented by fluorescein angiography. The National Eye Institute Visual Function Questionnarie 25 (NEI VFQ-25) was administered at baseline and at 12 months.

4.3.2 Inclusion criteria

To be eligible for the study the patient had to meet the following inclusion criteria:

1. age 50 years or older
2. occult CNV in the geometric centre of the fovea
3. lesion size less than 5000 µm in the greatest linear diameter (GLD)
4. best corrected visual acuity (BCVA) corresponding to a Snellen equivalent of 20/200 to 25/25.
5. clear ocular media

4.3.3 Follow up

At baseline and at every follow up (6, 12, 18, 24, 36 and 48 weeks), BVCA was measured with the Early Treatment Diabetic Retinopathy Study (ETDRS) chart, lesion size on fluorescein angiography and foveal thickness with optical coherence tomography (OCT).

4.3.4 Primary outcome measure

The primary outcome measure was the proportion of patients who lost fewer than 15 EDTRS letters at 12 and 24 months follow up (paper 3). The result of patient reported vision-related function was conducted in the same set of patients (paper 4).

4.3.5 Secondary outcome measures

Secondary outcome measures included the proportion of patients who gained 0 letters or more, the change in mean lesion size and GLD and the change in foveal thickness at 12 months follow-up.

5 RESULTS

5.1 SUBTHRESHOLD TTT AS A TREATMENT OF EXPERIMENTAL CNV IN THE MOUSE (PAPER 1)

Increasing the TTT laser power yielded gradually more visible effects. When using a power setting of 50 mW (subthreshold) no histological damage was seen in the neural retina, RPE, or choroid. By contrast, eyes treated with higher power exhibited progressively more damage to the neural retina. When TTT was applied to laser-induced CNV lesions, the height of the lesions was significantly reduced in response to all three power settings. The overlaying neural retina showed no apparent damage when subthreshold doses were used, whereas an outer nuclear layer disruption occurred with a power of 80 mW. Transmission electron microscopy confirmed that subtreshold TTT effectively occluded newly formed vessels.

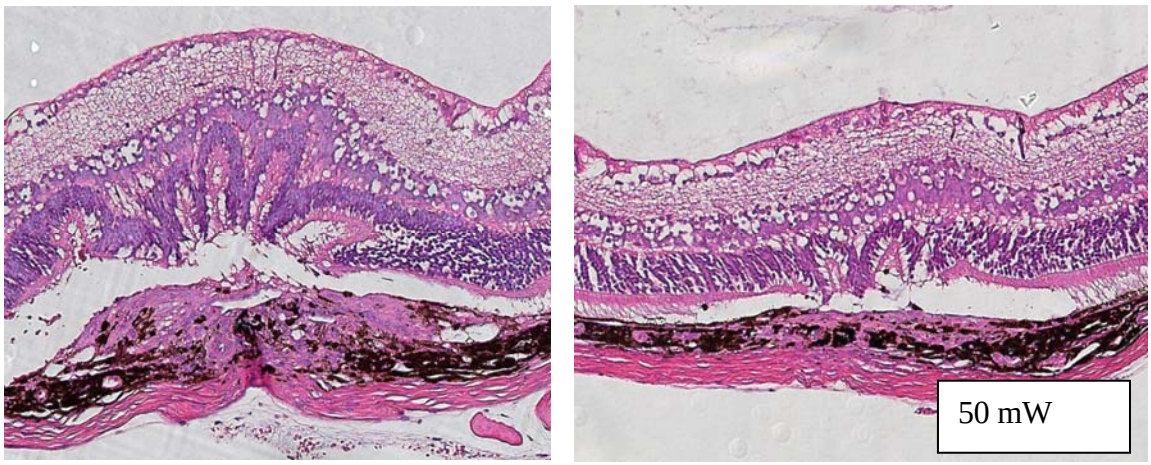

Figure 5. Light microscopy at 13 days after krypton laser coagulation showing a dome-shaped subretinal CNV complex.. After TTT treatment with a power of 50 mW, a marked thinning and fibrosis of the CNV membrane was seen without apparent damage to the overlying neural retina.

5.2 PDT OF EXPERIMENTAL CNV IN THE MOUSE (PAPER 2)

In normal eyes, increasing the light doses yielded gradually more damage to the neural retina and to the surrounding retina. When PDT was applied over laser-induced CNV lesions, the relative height and size of the lesions was significantly reduced using all

laser doses. Transmission electron microscopy one day after PDT treatment revealed an acute occlusion of many of the CNV vessels. At one week after PDT treatment patent vessels in the CNV lesion could still be detected irrespective of light dose applied.

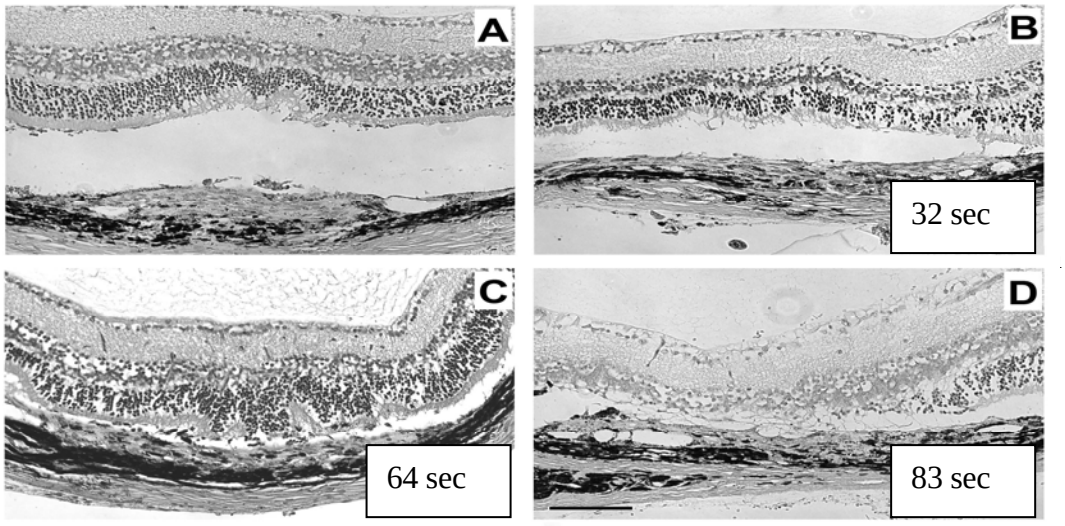

Figure 6. Light microscopy at 13 days after krypton laser treatment showing a dome-shaped subretinal CNV complex (A). After PDT treatment with light doses of 32 s (B), 64 s (C) and 83 s (D), a marked thinning of the CNV complex was seen. In addition to the regression of the CNV complex a disruption of the outer nuclear layer was seen with a light dose of 83s.

5.3 A PROSPECTIVE RANDOMISED STUDY ON LOW-DOSE TTT VERSUS PDT FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION (PAPER 3)

The percent of patients losing fewer than 15 ETDRS letters at 12 months was 75.0% in the TTT group and 73.9% in the PDT group. The percent of patients with preserved or improved BCVA was 36.5% in the TTT group versus 23.9% in the PDT group. The mean decrease in foveal thickness was 15% for TTT and 24% for PDT treated patients and the mean increase in total lesion area was -0.7% and -1.1% respectively. None of these differences were found to be statistically significant.

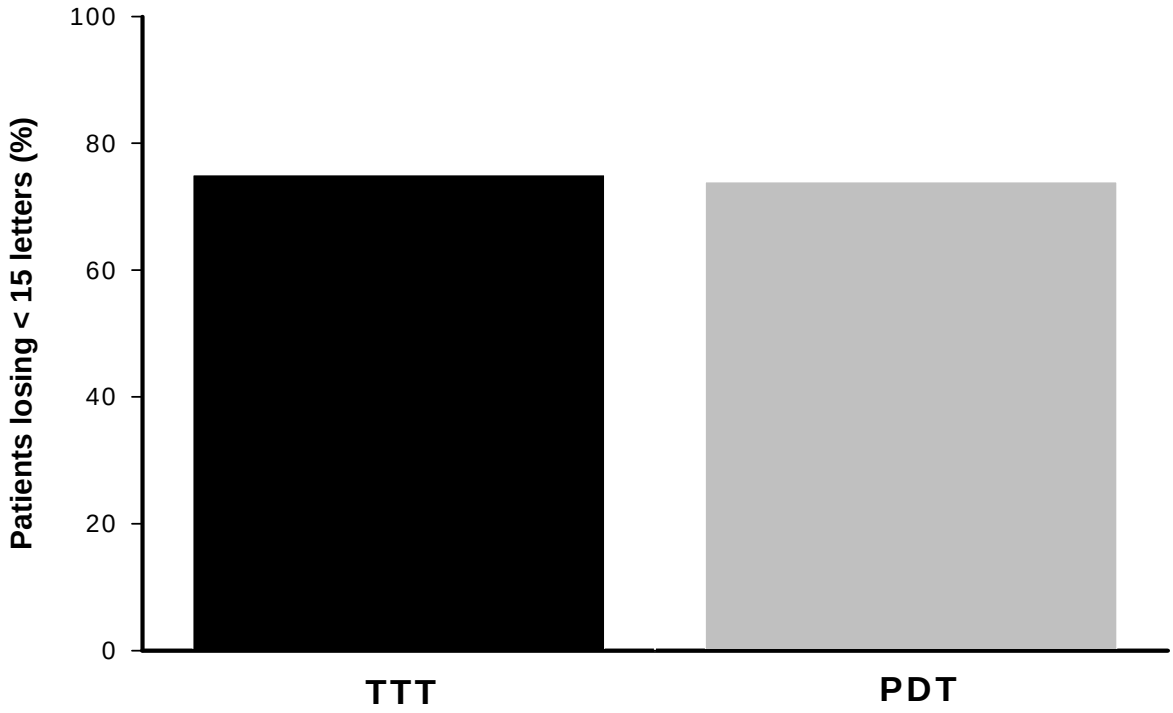

Figure 7. Primary outcome measure. The portion of patients with stabilised visual acuity was 75.0% and 73.9% in the TTT and PDT groups respectively. p>0.05

5.4 VISION-RELATED FUNCTION AFTER LOW DOSE TTT VERSUS PDT FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION (PAPER 4).

The NEI VFQ-25 questionnaire was used to measure the patient reported assessment of vision-related functions. The main outcome measure of the study, i.e. the proportion of patients with stabilized visual acuity, was similar between the TTT and PDT treated group.

80.1% (TTT) and 80.0% (PDT) patients, completed the NEI VFQ-25 at the 12 months follow up and the mean change in the score results was +1.2 for the TTT group and + 0.7 for PDT group (p>0.05)

None of the subscales categories showed significant differences between treatment groups. The VFQ-25 scores were lower in patients treated in their better seeing eye.

6 DISCUSSION

6.1 PDT VERSUS TTT FOR CHOROIDAL NEOVASCULARIZATION.

Several randomised, large-scale, trials have shown that PDT with verteporfin stabilises visual acuity in patients with classic, predominantly and certain types of occult lesions (TAP report 2, 2001; VIP 2 study, 2001). Regarding lesions displaying occult CNV, the positive effect of PDT apperared greater in patients with either smaller lesions (< 4 MPS disc areas), or lower level of visual acuity approximately (< 20/50) in the affected eye (VIP 2 study, 2001, VIP 1 study, 2003).

The desired effect of PDT is to cause a permanent vascular occlusion within the CNV lesion without damage to the surrounding tissue including the overlying neural retina (Miller et al 1995). However, many patients need more than one PDT treatment to stop the CNV leakage, and despite successful control of the leakage, most lesions will continue to grow (Schmidt-Erfurth 2000).

There are a few studies suggesting continued angiogenesis after PDT that in turn will lead to decreased visual acutiy, although at a lower rate than without treatment. For instance, an upregulation of VEGF and VEGF receptors has been demonstrated in choroidal endothelial cells post-PDT (Gelisken et al 2004, Grisanti et al 2004, Moshfeghi et al 2003, Schnurrbusch et al 2001, Schmidt-Erfurth et al 2003).

Regarding TTT, it has been assumed that as long as no biomicroscopically detectable retinal whitening is seen (i.e. subthreshold effects), only limited damage to the neural retina can occur. However, although several studies have indicated that TTT may successfully treat CNV in patients with wet AMD, resulting in a resolution of CNV without apparent retinal complication (Reichel et al 1999, Newsom et al 2001, Algvere et al 2001, Algvere et al 2003, Thach et al 2003), there have also been reports showing RPE damage after TTT (Thomson et al 2001, Salinas-Alamán et al 2003).

TTT is further limited by the fact that no controlled studies have been conducted to address its potential efficacy and safety.

PDT and TTT represent two different treatments of neovascular AMD with some similar but for the most part radically different features. For this reason, a head-to-head comparison of the two treatments in both experimental and clinical settings was performed in the current thesis.

6.2 SUBTHRESHOLD TTT AS A TREATMENT OF EXPERIMENTAL CNV IN THE MOUSE (PAPER 1)

Our experiments demonstrate that, indeed, no histological damage to the neural retina was induced with a laser power that was biomicrosopically invisible, i.e. assessed to be subtreshold.

Increasing the laser power resulted in gradually more visible effects in the retina and increasing histological damage.

Following TTT targeted to experimental CNV regression of the neovascular complex was seen without apparent damage to surrounding structures. Furthermore, transmission electron microscopy confirmed the presence of acute vascular occlusion that persisted for at least 7 days. Together this strengthens the theoretical basis for the mode of TTT action, namely that a low temperature increase can cause subretinal vascular occlusion. The results also underscore the importance of using subtreshold doses of TTT to avoid damage to the surrounding neural retina.

6.3 PDT OF EXPERIMENTAL CNV IN THE MOUSE (PAPER 2)

In this paper we confirm that PDT of CNV may create an acute occlusion of neovessels and an inhibition of CNV lesion growth without apparent injury to the surrounding neural retina.

However, as with TTT, a small increase in treatment dose may result in significant collateral damage. Moreover, ultrastructural analysis of PDT-treated CNV showed that treated areas will remain vascularized to some extent even at high light doses.

The fact that PDT did not cause permanent vascular closure of the CNV lesion suggests that treated lesions may still continue to grow. Thus, arrested CNV growth is not dose-dependent within the light dose ranges used herein. The basis for the subsequent revascularization after PDT may be due to recanalization of the occluded vessels, continued angiogenesis, or a combination of both events.

Analysis of choroidal flat-mount preparations after PDT confirmed that the CNV lesions continue to grow although at a slower rate than untreated lesions. Together, these results suggest that active angiogenesis remains after PDT but do not rule out the participation of recanalized vessels or the presence of vessels that escaped PDT.

6.4 A PROSPECTIVE RANDOMISED STUDY ON LOW-DOSE TTT VS PDT FOR NEOVASCULAR AGE-RELATED MACULAR DEGENERATION (PAPER 3)

The clinical experience indicates that the TTT radiation power is crucial for avoiding neural retinal damage. Furthermore, experimental experience indicates that the therapeutic window of TTT is narrow.

Therefore, in the present clinical study, the laser power was reduced to 136 mW/mm as compared to 248 mW/mm used in most previous studies.

Using this setting, TTT and PDT yielded visual results that were almost identical, and the results remained the same over time.

Although the main outcome measure was not met in our study, the similar outcome between the two groups suggests that any difference found would probably be small and not clinically relevant. The result is not surprising since there seems to be considerable similarities between the pathophysiological responses after TTT and PDT.

In human eyes with neovascular AMD TTT has been associated with transient decreased blood flow 24 hours after treatment, but the decrease was not sustained (Ciulla et al 2001). Studies have also indicated that the focal ERG amplitude decreases transiently during TTT (Falsini et al 2003). These results are also supported by a transient decline in visual acuity after TTT (Robertson&Salomao 2002).

In contrast, a transiently increased vascular leakage was demonstrated on angiography within one hour after TTT. Similary, PDT caused immediate thrombosis (Zacks et al 2002, Criswell et al 2005) and also initially a breakdown of vascular barriers (Michels&Schmidt-Erfurth 2003). In patients treated with PDT angiography has indicated a hypoflourescent area corresponding to the laser spot and closure of choroidal vessels proceeding for as long as one week (Michels et al 2003), and an upregulation of VEGF and VEGF receptors has been demonstrated indicating a rebound of angiogenesis (Schmidt-Erfurth et al 2003).

6.5 VISION-RELATED FUNCTION AFTER LOW-DOSE TTT VS PDT FOR NEOVASCULAR AMD (PAPER 4)

The impact of vision on the quality-of-life is often inadequately reflected by traditional clinical measurements and descepancies has been found, i.e. an improvement in visual acuity is not always reflected in a better perception of visual function.

In the clinical study on TTT and PDT, we monitored the patient reported vision-related function.

The mean change in the NEI VFQ-25 score indicated a small improvement in both treatment groups. We did not include a placebo group and therefore cannot evaluate whether either treatment resulted in a meaningful change on patient-reported visual function.

Previous studies have shown that a 10-point change in the NEI-VFQ-25 score roughly corresponds to a 15 letter change in visual acuity (Miskala et al 2003). There was no difference in patient-reported visual function between treatment groups. This was expected, since the visual outcomes were virtually identical for the TTT and PDT treated patients. Thus, our results on patient-reported visual function and visual acuity are in overall agreement.

7 CONCLUDING REMARKS

The main outcome measure was not met in the clinical study, e.g. that low-dose TTT would prove superior to PDT at stabilising BCVA. Although the study was not powered to detect non-inferiority differences it is likely that low dose TTT and PDT are equipotent in the set of patients studied. This is supported by the experimental results presented demonstrating that both treatments can significantly reduce CNV without apparent damage to surrounding structures.

There are however two notable differences between the treatments. On the one hand, in the clinical situation it is easier to titrate the optimal dose of PDT. On the other hand, from the experimental data we observed that only PDT (and not TTT) treated CNV lesions showed a tendency to revascularize.

With the advent of intravitreal anti-VEGF compounds neither PDT nor TTT are first-line treatments for neovascular AMD. However, there are several limitations to anti-VEGF therapy including a very high cost and the need for repeated intravitreal injections. In order to reduce the burden of this treatment on the patient as well as on the healthcare system, the development of strategies aimed at reducing the number of intravitreal injections will be critical.

Combination therapy is one such possibility. Our present findings indicate that low-dose TTT is equipotent to PDT. Considering the extremely low cost of TTT, it should make an attractive adjuvant therapy to intravitreal pharmacotherapy. In particular, a successful combination strategy with low-dose TTT and the reasonably cheap anti-VEGF compound bevacizumab (Avastin®) would be a “dream-team” in neovascular AMD management addressing both the economic and logistic problems. However, this concept needs to be further investigated in randomized clinical trials.

8 POPULÄRVETENSKAPLIG SAMMANFATTNING

Åldersrelaterad makuladegeneration (AMD) utgör den vanligaste orsaken till synnedsättning och blindhet i Sverige liksom i övriga västvärlden.

Kärlnybildning (chorioidal neovaskularisation, CNV) under makula (gula fläcken) ses vid sk våt AMD, den allvarligaste formen av AMD. Prognosen vid CNV är dålig då de nybildade kärlen kan ge upphov till blödningar, vilket snabbt kan leda till en svår synnedsättning.

Synnedsättningen vid våt AMD har sedan några år kunnat bromsas med fotodynamisk terapi (PDT). Vid PDT injiceras ett ljuskänsligt färgämne i ett kärl i armen, detta ämne ansamlas i de nybildade kärlen och aktiveras av ett svagt laserljus. En sk fotokemisk reaktion sätts igång vilken leder till en proppbildning och avgränsad förstörelse av de nybildade kärlen.

Transpupillär termoterapi (TTT) är en teknik där en temperaturförhöjning på ca 10 C^o, åstadkommes genom att en laserstråle riktas mot näthinnan. Den förhöjda temperaturen ger en proppbildning i nybildade kärl utan att ljuskänsliga ämnen behöver ges.

Flera mindre rapporter har indikerat att TTT kan användas för behandling av CNV utan risk för lokala komplikationer men större kontrollerade studier saknas. Andra studier har dessutom påtalat en potentiell risk för över/underbehandling med konsekvenser i form av permanenta skador på näthinnan, alternativt utebliven effekt på kärlnybildningen.
Resultaten av studierna i avhandlingen visar att både PDT och TTT reducerar kärlnybildning på experimentell nivå, utan påverkan på omgivande vävnader. I båda fallen såg man däremot en skada på omgivande näthinna när behandlingseffekten ökade vilken i sin tur innebär att det terapeutiska fönstret är smalt för såväl PDT som TTT.
En viktig skillnad i effekt kunde noteras mellan PDT och TTT. Såväl PDT som TTT gav en omedelbar proppbildning i områden med kärlnybildning, men efter PDT öppnade sig kärlen igen, medan ocklusionen efter TTT kvarstod i upp till en vecka.

Avhandlingen innefattar även en randomiserad klinisk studie på 98 patienter med våt AMD vilka fått antingen TTT eller PDT under 12 månader. Med stöd från våra experimentella data, vilka visade att det föreligger risk för skada på omgivande vävnad med TTT, användes i studien den lägre dosen 136mW/mm istället för den högre dosen 268mW/mm som använts i de flesta tidigare studier.

Resultatet av studien visar att effekten på synförmågan var nästan identisk mellan TTT och PDT. I båda fallen skedde en stabilisering av synskärpan hos ungefär 75 % av patienterna. Majoriteten av patienterna i studien upplevde även stabilisering av synrelaterad livskvalité. Inte heller här förelåg det någon skillnad mellan TTT och PDT.

Helt nyligen har en ny behandling med överlägsna resultat på synskärpa och patientupplevd synkvalité introducerats vid våt AMD. Behandlingen innebär att ett kärlhämmande läkemedel (anti-VEGF) ges som upprepade injektioner i ögats glaskropp. Anti-VEGF behandling är mycket dyr, resurskrävande och det finns en risk för intraokulära komplikationer. Utveckling av nya behandlingsstrategier vid våt AMD har därför hög prioritet även i framtiden. En tänkbar sådan utveckling är kombinationsterapi mellan t.ex. anti-VEGF och PDT.

Då resultaten av denna avhandling visar att effekten av TTT är jämförbar med PDT skulle TTT i kombination med anti-VEGF kunna utgöra ett kostnadseffektivt alternativ till dagens behandling av våt AMD.

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