FREE RUNNING NEODYMIUM-YAG LASER COAGULATION OF THE HUMAN FOVEA

A Light and Electron Microscopic Study

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Abstract: Free Running Neodymium-Yttrium Aluminum Garnet (FR Nd-YAG) laser coagulation of the macula was performed in seven human eyes. Follow-up varied from 1 to 15 days. Damage to the outer retinal layers was apparent immediately after exposure whilst that in the inner retinal layers developed subsequently. The choriocapillaris and major choroidal vessels were occluded. Visual acuity changed in an unpredictable way. RETINA 9:312–318, 1989

Disciform macular degeneration is still a sight-threatening disorder despite recent advances in laser therapy. It has been estimated that of all patients with senile disciform macular degeneration less than 20% were suitable for laser treatment. Neovascular membranes under the fovea cannot be treated without endangering the function of the inner and outer retina. Due to the absorption of light energy by hemoglobin, xanthophyll pigment in the macular area and melanin in the retinal pigment epithelium (RPE) most lasers used for coagulation of subretinal neovascular membranes damage the outer retina. Damage to the inner retina may be restricted by the use of green argon, red krypton and dye lasers with wavelengths that are only minimally absorbed by the xanthophyll pigment. On theoretical grounds it has been postulated that Continuous Wave (CW) Nd-YAG laser light with wavelength of 1064 nm might be safer for the inner retina when used in coagulating subretinal parafoveal neovascular membranes than argon green or krypton red lasers. Experiments in monkeys have shown that the CW Nd-YAG laser effect is mainly restricted to energy absorption in the RPE, and adjacent outer retina layer and choriocapillaris.

The purpose of this paper is to describe the effects of FR Nd-YAG photocoagulation on the human macula in order to determine its suitability for possible treatment of subretinal parafoveal neovascular membranes.

Patient Selection and Methods

Patients with an eye requiring enucleation for a malignant melanoma were asked to cooperate in this study. The protocol used had the approval of the ethical committee of the Eye Hospital and is in accordance with the Helsinki Convention. Patients were fully informed about the nature of the investigation and were warned about the possibility of central vision loss prior to enucleation.

As part of another study patients received during
the two days prior to enucleation 8 Gy of external irradiation to the affected eye by means of a betatron electron source. The study protocol stated that the timing of enucleation was not to be delayed for the purpose of the laser experiments. As soon as patient’s consent was obtained FR Nd-YAG coagulation was applied. The interval between laser coagulation and enucleation was solely determined by the time necessary for arranging the betatron irradiation.

The Sirius-Microruptor II (Lasag, Thun, Switzerland) was used as a FR Nd-YAG laser source. The energy of every coagulation was recorded using a printer coupled to the laser. The pupil was dilated, the cornea anesthetized, and coagulations were applied through a Goldmann three mirror contact lens (Haag Streit, Bern, Switzerland). The standardized coagulation time was 10 msec with a spot size 100 μm in air. The coagulation energy was such as to produce a moderate whitish coagulation effect. This was determined by focussing the laser beam near the temporal arcade. Once this energy level was established multiple discrete coagulations were placed cross-shaped in the center of the posterior pole (Fig. 1). Care was taken to avoid the clinically visible part of the tumor which meant that in some cases foveolar coagulations could not be applied. Pulsed laser coagulations were not used in order to avoid the risk of metastasis due to shock waves.

Best corrected visual acuity was recorded before laser coagulation and subsequently on the day prior to enucleation. Color photography was performed immediately after coagulation and on the day before enucleation.

Immediately after enucleation an equatorial scleral incision was made and the eyes were immersed in a mixture of glutaraldehyde (1.25%) and paraformaldehyde (1%) in cacodylate buffer (0.08M) at pH 7.4. After several days fixation the melanoma containing part of the eye was taken by the pathologist. Individual laser coagulations were located and coded. For transmission electron microscopy (TEM) tissue was postfixed with OsO₄ and routinely embedded in Epon 812 (Merck, Darmstadt, W. Germany). The Epon-embedded coagulations were serially sectioned at 1 μm and these semithin sections were stained with toluidine blue and examined by light microscopy. Once the largest diameter of the coagulation had been identified ultrathin sections were cut, stained with uranyl acetate and lead citrate and studied in a Philips EM 400 electron microscope. The scanning electron microscopic (SEM) procedure consisted of thorough rinsing in buffer solution followed by dehydration in a graded series of ethanol, critical point drying with CO₂ and gold coating. The SEM specimens were examined using a Philips SEM 505 (Philips, Eindhoven, The Netherlands).

**Fig. 1.** FR Nd-YAG laser coagulations of human retina. A, Case 5, 15 min. after coagulation. Central coagulations seem more intense although energy per coagulation varied only between 0.33 and 0.36J. Note slight halo around foveal coagulation. B, Case 5, 14 days after coagulation. Around the pigmented coagulation there is an area of depigmentation. Arrow indicates coagulation in Fig. 4.

**Results**

Seven patients, aged between 31 and 71 years (Table 1) took part in the study. The diagnosis of melanoma diagnosis was confirmed histologically in all eyes. The laser energy necessary to obtain moderate white coagulations and the changes in visual acuity are given in Table 1. In two cases (6 and 8) the laser coagulations could not be recovered after that part of the eye containing the melanoma had been removed. In case 6 small intraretinal hemorrhages and haloes were seen around the laser spots 15 minutes after coagulation.

**Histology and Electron Microscopy of Laser Burns**

Despite the variation in laser energy and time to enucleation, the light and electron microscopic observations in the inner and outer retina as in the choroid can be summarized as follows.

One day after extrafoveal coagulation (Fig. 2) all constituents of the inner retinal layers showed
Table 1. Laser Energy Necessary to Obtain Moderate White Coagulations in Seven Patients

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yrs.)</th>
<th>VA Before Laser</th>
<th>VA 1 Day Before Enucleation</th>
<th>Interval Laser Enucleation (days)</th>
<th>Mean Energy Laser Burns (Joules)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
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<td>fc</td>
<td>fc</td>
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<tr>
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<td>7</td>
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<tr>
<td>4</td>
<td>55</td>
<td>1.0</td>
<td>0.5</td>
<td>9</td>
<td>0.63</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
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<td>0.15</td>
<td>15</td>
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</tr>
<tr>
<td>6</td>
<td>31</td>
<td>hm</td>
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<tr>
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mild edema on light microscopy. Focal pyknosis of the outer nuclear layer was visible as darkly staining nuclei by light microscopy and as condensed heterochromatin and partly disrupted nuclear membranes by electron microscopy. Small vacuoles were present in the photoreceptor layer. Evidence of subretinal exudate was noted with numerous vacuoles of varying size surrounding the disrupted RPE. A small hemorrhage in the choroid was seen.

The edematous changes persisted for as long as 15 days. A large vacoule with adjacent smaller ones were observed in the area between the inner nuclear and outer plexiform layer after nine days (Fig. 3). Disruption of the inner limiting membrane both within the coagulated area and outside was seen in cases 4 and 5 after nine or more days follow up. Disruption of the medial limiting membrane was present in all cases. Pyknotic changes, visible as darkly staining nuclei in the inner and outer nuclear layer were present after 9 and 15 days (Figs. 3, 4).

The most severe effects of laser coagulation were found in the outer retinal layers six or more days after coagulation. These showed pyknosis and reduction of the number of nuclei of the outer nuclear layer as well as destruction and disappearance of the inner and outer segments of the rods and cones (Figs. 3, 4). Subretinal edema was present and this area contained large cells filled with numerous melanosomes (Figs. 4, 5). Disruption, vaccuolization and hypertrophy of the RPE was well marked. These changes were present in all cases but more apparent in cases 2 to 5 in which six or more days had elapsed between coagulation and

Fig. 2. Case 1. Extrafoveal coagulation. 1 day. Slight edema in inner retina (IR). Note pyknosis between bold arrows of outer nuclear layer and damage of photoreceptors in the outer retina (OR). Subretinal exudate and vacuoles. Disruption of the RPE (arrowheads) and hemorrhages in choriocapillaris and choroid (CH). LM, 1 µm toluidine blue section.
Fig. 3. Case 4, 9 days. Parafovea burn. Note large vacuole in inner nuclear layer (INL) extending into outer plexiform layer (OPL); loss of photoreceptors and decreased number of nuclei in the outer nuclear layer (between small arrows) and destruction of the RPE (bold arrows). Numerous melanin containing giant cells in the disrupted region. The choroid was peeled off during the preparation. Breaks in inner limiting membrane are seen both inside and outside the coagulated areas. LM, 1 μm toluidine blue section.

Fig. 4. A. Case 5, 15 days. Foveolar coagulation. Pyknosis of nuclei in the inner and outer nuclear layer, loss of nuclei in the outer nuclear layer and disappearance of photoreceptors. B. Rupture in Bruch’s membrane (between asterisks) with multinucleated giant cells (GC) containing melanin and ingrowth of fibroblasts (arrowheads). LM, 1 μm toluidine blue section.

enucleation. Reconstruction at the RPE level could be observed after 15 days where a monolayer of fibroblasts was seen (Fig. 4).

In case 3, seven days after laser coagulation, part of the outer nuclear layer was atrophic due to disappearance of pyknotic nuclei. Structures that most likely were empty encasings of the photoreceptors, partly torn from the area of the external limiting membrane, were present around this area. The subretinal exudate contained disrupted RPE cells (Fig. 5).

In most cases, vacuolization and edema were found between the RPE and Bruch’s membrane. This edema increased with time to enucleation. On light microscopy the structure of Bruch’s membrane was relatively normal although the ageing processes of thickening and calcification were seen. In case 4 a distinct basal laminar deposit containing small vacuoles was noted in the coagulated area. On transmission EM vesicular and tubular structures compatible with ageing changes were seen. Disruption of Bruch’s membrane was only an incidental finding. In the case enucleated 15 days after coagulation a multinucleated giant cell containing melanin was found crossing a gap in Bruch’s membrane (Fig. 4).

In all cases the area of the coagulation was characterized by choriocapillaris with markedly swollen endothelium and lumen occlusion. Sattler’s and Haller’s layers of the choroid were edematous and swollen and occluded arteries and veins as well as hemorrhages were observed in all cases. The severity of these changes was maximal in the case enucleated
nine days after coagulation and was less pronounced in the case enucleated at day 15. The normal nature of structures outside the coagulated areas excluded artifact as the basis for these changes.

In SEM specimens from case 5 mounds of necrotic pigment epithelial cells adherent to the retinal pigment epithelium were visible at the coagulation sites (Fig. 6). On semithin sections these mounds were found to contain multinucleated giant cells filled with melanosomes.

Discussion

Both FR and CW Nd-YAG lasers work in the thermal mode. Free running lasing implies that lasing occurs during that time when the pumping source in the laser cavity is excited. In case of the Nd-YAG laser this means during the time the pumping flash lamp is ignited and this happens only intermittently. In a CW laser the output is essentially constant with time as the pumping goes on continuously. In practice
the difference between FR and CW YAG lasers is that the exposure time in the former is 10 to 20 msec and up to several minutes in the latter.

The FR Nd-YAG laser mode was used in this study because of its long wavelength and because these lasers are in contrast to the CW mode widely available. The FR mode has been used for coagulation of diabetic retinopathy. Apart from less absorption by macular xanthophyll, the advantage of a long (1064nm) rather than a short (eg. 530nm, green) wavelength is the higher absorption by oxyhemoglobin and the lower scatter in the ocular media.

The exposure duration of 10 msec was very short. FR Nd-YAG coagulations with exposure times of 10 or 20 msec resulted in more or less the same damage to the outer retina and choroid, as CW Nd-YAG coagulations of 700 msec or longer duration. As we will see later the incidence of hemorrhages was much smaller with the longer coagulation times and was the only difference noted.

An initial attempt to determine threshold exposure levels was abandoned as a marked difference of reaction was found between the eyes. This was probably due to differences in absorption by the ocular media and in pigmentation of the choroid. The intensity of the coagulations was less than that usually applied to eyes with subretinal neovascular membranes. In case 6 halos were seen around the coagulations and intraretinal hemorrhages were also seen a few minutes after laser coagulation, indicating that the energy was too high. As this patient was the youngest studied it might be that less absorption of energy by the media was the cause for this. The effect during coagulation seemed the same as with the other cases and the mean energy was in the lower range (0.38 J) in comparison with the others. Unfortunately we could not recover the coagulation sites of the retina after the pathologist had removed the posterior part of the eye.

The incidence of hemorrhages produced by FR Nd-YAG coagulations of the retina varied in rabbits between 10% and 50% and has been a matter of discussion between investigators. A focused laser beam would give less hemorrhages than an unfocused one. The shorter the pulse duration, the greater the chance of hemorrhages. Specifically for FR Nd-YAG lasers there may be a considerable variation in the emission pattern of the pulse-peak between one apparatus and the other. The steeper the rise in pulse energy, the more chance of hemorrhages. From the fact that hemorrhages were only present in one out of seven cases one would conclude that our FR Nd-YAG laser emits a pulse with a slowly rising peak energy, thus creating little thermal expansion of the tissue. It is probable that a low-power CW Nd-YAG laser with exposure times of 0.5 to 5 seconds would be less likely to cause hemorrhages.

We ruled out the possibility of artifacts in the histological slides by comparing coagulated areas with adjacent non-coagulated areas in the same slide. Thus in Figure 3 the large vacuole might have at its lower right corner a small artificial gap but the vacuole itself seemed to be genuine. Also in Figure 4 the possibility of obliquely cutting the receptor encasings was excluded by the normal orientation of the surrounding retina in the slide. The scleral incision immediately after enucleation allowed the fixative to enter the eye rapidly so that the findings in the choriocapillaris and choroid are unlikely to be artifacts. Only in case 4 were breaks in the inner limiting membrane seen both inside and outside the coagulated areas (Fig. 3). Perforation of the inner limiting membrane has been described as resulting from laser coagulations with a pulse energy between 3.6 and 50mJ. The disruption therefore might be interpreted as genuine but artifacts cannot be ruled out.

Our histological results are in general in concordance with previous animal studies with different laser modalities. An advantage of animal over human studies is that the laser effects can be studied quantitatively over a longer period of time in comparable animals. Due to differences in patients ages and the amounts of applied laser energy it is hard to tell if the laser effect after 1 or 10 days would be the same for all cases. Slight differences in the severity of endothelial swelling in the choriocapillaris, for instance, might be attributed to differences in wavelength, energy and coagulation or survival time. The amount of repair in the RPE and choriocapillaris as described in rabbits after two weeks could not be found in this study. This may be due to differences in retinal structure, faster reparative processes in rabbits or their relatively younger age. Despite a careful search we found ruptures in Bruch's membrane with fibroblasts only in case 5. RPE cells and melanin containing multinucleated giant cells were seen migrating through Bruch's membrane towards the choriocapillaris (Fig. 4). Most human eyes with experimental laser coagulation were enucleated 24 hours after the coagulation thus hampering exact comparison. The only case with six days survival showed partial obstruction of choriocapillaries and pigment epithelium regeneration.

On the SEM picture of a laser burn (Fig. 6) a necrotic mound was visible. On higher magnification multinucleated giant cells with melanosomes seemed to stick to the RPE. At the base of the mound fenestrations in the RPE had disappeared (Fig. 6, bold arrow). It seems likely that the photoreceptors in and
around this mound were considerably damaged. In the rabbit regeneration of photoreceptors has been found four weeks after 532 nm pulsed YAG coagulations.\textsuperscript{16} In our material only regrowth of the RPE was visible. The destruction of the interphotoreceptor matrix visible in Figure 5 makes it unlikely that even with longer follow-up photoreceptors in the human will regenerate in a useful pattern.

As far as we know the absorption spectrum of xanthophyll has only been studied in the visible light range. It was found that the absorption decreased from 85% at 460 nm to 15% at 540 nm.\textsuperscript{17} One would assume that the Nd-YAG with 1063 nm would be poorly absorbed. Nevertheless there was mild damage in the inner retinal layers that became more pronounced in the cases followed for six or more days before enucleation. In all cases the outer retina was severely injured so that for clinical purposes there seems to be no great advantage over the krypton or green argon laser.

In conclusion FR Nd-YAG coagulation of the human fovea and macular area with 10 msec, 100 μm spot size and energies between 0.24 and 2.45 Joules leads after six or more days to marked inner retinal damage. Severe disruption of the outer retina and of the RPE were present one day after the coagulation with laser settings less intense than those used for closure of subretinal neovascular membranes with the green argon laser. In all cases closure of the choriocapillaris was present. As a result it is concluded that the outer retinal damage probably will remain the limiting factor with regard to FR Nd-YAG photocoagulation of subretinal membranes encroaching on the foveola.

**Key words:** human macula, FR Nd-YAG laser, retina.

**References**


