NEW THERAPEUTIC MODALITIES FOR LASER RETINAL INJURY

ANNUAL REPORT

TIM T. LAM
MARK O.M. TSO

MARCH 29, 1991

Supported by
U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21702-5012

Grant No. DAMD17-89-Z-9025

University of Illinois
833 S. Wood Street
Chicago, Illinois 60612

Approved for public release; distribution unlimited.

The findings in this report are not to be construed as an official Department of the Army position unless so designated by other authorized documents.
DISCLAIMER NOTICE

THIS DOCUMENT IS BEST QUALITY AVAILABLE. THE COPY FURNISHED TO DTIC CONTAINED A SIGNIFICANT NUMBER OF PAGES WHICH DO NOT REPRODUCE LEGIBLY.
Two grades (II and III) of retinal lesions were evaluated using clinical, histopathologic, and morphometric methods from 3 days up to 20 days after the laser insult. Morphometric parameters varied according to time and the energy of the laser used. Preliminary evaluation of the efficacy of high-dose, and continuous infusion of methylprednisolone was performed using a swivel-tethering system. Clinical study at 3 and 10 days after laser injury suggested a dramatic beneficial effect when the drug was given at 24 hours before injury and continued for 4 days. Preliminary histopathologic observation also suggested a protective effect of the treatment regimen. Morphometric analysis is underway. In addition, two human cases of laser injury to the retina were studied clinically as a basis for clinical application of therapeutic modalities.
Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the U.S. Army.

N/A Where copyrighted material is quoted, permission has been obtained to use such material.

N/A Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

N/A Citations of commercial organizations and trade names in this report do not constitute an official Department of the Army endorsement or approval of the products or services of these organizations.

Yes In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

N/A For the protection of human subjects, the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>FOREWORD</td>
<td>i</td>
</tr>
<tr>
<td>INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>METHODS</td>
<td>4</td>
</tr>
<tr>
<td>RESULTS</td>
<td>6</td>
</tr>
<tr>
<td>CONCLUSION</td>
<td>10</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>31</td>
</tr>
</tbody>
</table>

## DIAGRAMS AND FIGURES

| Diagram 1. | Measurements of outer nuclear layer (ONL) gap (A) and the width of the depigmented RPE (B) in laser-induced retinal lesion (Grade III) | 12 |
| Diagram 2. | Continuous intravenous infusion system in primates | 13 |
| Diagram 3. | Commercially available tethering system with jacket and swivel | 14 |
| Figure 1.  | Fundus pictures of Grades II and III lesions at 3 days after induced injury | 15 |
| Figure 2.  | Fundus pictures of Grades II and III lesions at 10 days after laser injury | 16 |
| Figure 3.  | Fluorescein angiograms of Grades II and III lesions at 3 days | 17 |
| Figure 4.  | Fluorescein angiogram of Grades II and III lesions at 10 days after injury | 18 |
| Figure 5.  | Histopathologic features of Grade II lesions at (A) 3, (B) 10, and (C) 20 days after laser injury | 19 |
| Figure 6.  | Histopathologic features of Grade III lesions at (A) 3, (B) 10, and (C) 20 days after laser injury | 21 |
Figure 7. Time dependent relationship of the outer nuclear gap and the retinal pigment epithelial depigmented gaps in Grade II lesions .................................................. 23

Figure 8. Energy dependent relationship of the outer nuclear layer gap and the retinal pigment epithelial gap ............................................................ 24

Figure 9. Fundus picture of Grades II and III retinal lesions at 3, and 10 days after laser injury in methylprednisolone-treated monkey ............................................. 25

Figure 10. Fluorescein angiogram of the methylprednisolone-treated laser lesions ................................................................. 27

Figure 11. Fundus picture of patient #1 at 3 months after the initial injury ...................................................... 29

Figure 12. Fundus picture of patient #2 at 3 months after laser injury ................................................................. 30
March 29, 1991

ANNUAL REPORT

New Therapeutic Modalities for Laser Retinal Injury
Research Grant DAMD 17-89-Z-9025

I. Introduction

The widespread use of lasers in modern weaponry and industrial settings have greatly increased the risk of ocular laser injury of military and civilian personnel. The incidence of ocular injury by laser in the general population is not known. Wolf\textsuperscript{1} reviewed 23 cases reported in the literature. In 1990, we studied two cases of accidental retinal injury by laser from our Macular Clinic. In one case, the patient appeared to have an earlier unreported accidental laser injury to the retina also. It appears that the occurrence of laser induced retinal injury in the general population may be higher than is perceived.

Retinal injury by laser is known to cause loss of vision, macular hole formation, neovascularization and hemorrhage. There have been few studies on the treatment of laser induced retinal injury partly due to the misconception that the injury is severe and treatment may be ineffective. Ishibashi et al\textsuperscript{2} reported the inhibition of subretinal neovascularization by dexamethasone in laser induced retinal lesions in monkeys. Belkin et al\textsuperscript{3} studied the use of urokinase treatment in laser induced vitreous hemorrhage in rabbits and concluded that urokinase did not accelerate the absorption of blood from the vitreous but prevented the development of severe vitreous fibrosis, which followed the hemorrhage in some of the control laser induced eyes.

Clinically, of the 23 patients reported by Wolf, ten had steroid therapy. Corticosteroids, including triamcinolone, prednisone, and corticotrophin, were given topically, intervascularly, or by retrobulbar injections. Eleven cases had
vitreous hemorrhage. However, there is no evidence to show the efficacy of these various treatments.

While it is generally believed that the primary pathogenetic mechanisms for laser induced retinal injury are mechanical and/or thermal, it is not known whether other factors may be involved. To develop a rational approach to the therapy of laser injury, we proposed to test the following hypotheses:

(1) Inflammation reactions play an important role in the excessive tissue reactions after retinal laser injury and hence anti-inflammatory agents such as corticosteroids may ameliorate the reparative processes in retinal laser injury;

(2) Free radicals have an important role in retinal laser injury, both in the initial injury and the following tissue reactions, and hence antioxidants may assist the healing processes.

Underlying the above hypotheses we also presume that there are secondary injuries or cell death after the primary laser injury. Our approach is to limit this secondary cell death or injury after the initial mechanical or thermal injury to the retina induced by laser. Our rationale for evaluating the efficacy of corticosteroids in laser induced retinal injury is based upon the following findings. (a) Close to 50% of the patients reported by Wolf had been given steroid therapy and in some cases, the physician reported improvement. Yet, these are uncontrolled studies. (b) Recent findings by Hall et al⁶⁻⁸ and others have established that high doses of methylprednisolone are effective in inhibiting lipid peroxidation, which is mediated by free radicals and believed to play an important role in cell membrane disruption and cell death in various injuries, including CNS injury. In addition, the clinical trial study reported in the May, 1990 issue of The New England Journal of Medicine⁹ showed a beneficial effect of high dose of methylprednisolone in acute spinal cord injury. This latest success in the use of high dose of methylprednisolone in the treatment of CNS injury¹⁰,¹¹ confirms the
hypothesis that lipid peroxidation by free radicals may play an important role in CNS neuronal tissue injury. Although dexamethasone has also been shown to be effective in inhibiting lipid peroxidation it possesses a potent glucocorticoid action. Furthermore, methylprednisolone has been extensively tested in various animal models of CNS injury and has been found to be effective. Based on our hypothesis that there is secondary cell death or injury after the primary laser injury in retinal tissues, and that inflammatory reactions and lipid peroxidation may play important roles in those secondary changes, we proposed to study the effect of high dose of methylprednisolone in laser induced retinal lesions.

This current contract seeks to establish new therapeutic modalities for retinal laser injury of varying severity. In particular, our goal for the past year was to evaluate the efficacy of corticosteroids in laser induced retinal lesions.

In order to accomplish the primary objective of this contract, we have developed the following:

1. To generate retinal laser lesions reproducibly.
2. To quantify those lesions.
3. To deliver the drug effectively.

In this reporting period, we have been able to generate retinal lesions with different severity reproducibly. In addition, we have developed quantitative means as indexes of the severity of injury. We have also been able to test and establish a reliable means of drug delivery to the primate. Our findings, at this stage, though preliminary, have suggested that high doses of methylprednisolone given prior to laser injury and continued up to three days after the injury show beneficial effects in ameliorating the retinal injury induced by lasers.

In preparation for possible clinical application of our experimental work in using pharmacological agents for laser retinal injury, we are following the clinical courses of patients with retinal injury induced by laser.
II. Methods

A. Experimental

Using clinical and histopathological criteria we divided retinal laser lesions into four grades: I, II, III, and IV. In Grade I lesions, only the retinal pigment epithelial layer is affected. In Grade II lesions, the retinal pigment epithelial layer as well as the outer nuclear layer are affected while in Grade III lesions, the retinal pigment epithelial layer, the outer and inner nuclear layers are all affected. In Grade IV lesions, vitreous hemorrhage is seen in addition to necrosis in all retinal layers. In this study, only Grades II and III lesions were reported. At least four lesions, each of Grades II, and III were inflicted in comparable areas of the retina in each of the six monkey eyes by an argon laser (Coherent Medical 920) with a slit lamp delivery system (Zeiss). A 300 micron spot size 0.1 second duration and energy settings at 0.25 mW (Grade II) or 0.7 mW (Grade III) were used. The clinical features of the laser lesions were followed by ophthalmoscopic examination and recorded by fundus photography and fluorescein angiography. The lesions were studied at 3, 10, and 20 days after injury. The eyes after enucleation were fixed in 4% paraformaldehyde and 1% gluteraldehyde, post fixed in Dalton's osmium fixative, dehydrated in alcohol, and embedded in epoxy resin. One micron serial sections were cut until the center of the lesion was reached and studied by light microscopy.

Morphometric index based on histopathological features were used to quantify the effectiveness of drug treatment. In order to quantify the degree of damage of the lesions, we measured the width of the disrupted photoreceptor layers and the width of depigmented RPE at the center of the lesions (see diagram 1).

Two drug administration systems were tested. The first system was developed by our animal facility personnel. Briefly, an intravenous catheter was
implanted in the arm of an animal. An intravenous tube was attached to the catheter and a stainless steel conduit was used to protect the tube. An orthoplastic tube was placed on top of the intravenous catheter, positioned and secured with adhesive tapes (see diagram 2 for illustration). The second system tested was a commercially available swivel-tethering system (Alice King Chatham Medical, Los Angeles, CA). This system is similar to the previous one but instead of an orthoplastic cast a jacket was used to protect the implanted catheter from being removed by the animal (see diagram 3 for illustration). For both systems continuous intravenous infusion of methylprednisolone was accomplished by the use of a syringe pump (Model 355, Sage Inst., Boston, MA). Using the first infusion system a bolus injection of methylprednisolone at 30 mg/kg was given to the animal immediately after laser injury. Continuous infusion of methylprednisolone at a dose of 5.4 mg/kg/hr was started at one hour after the laser injury and continued until the system was disrupted by the animals at 8 hours after laser injury. In the second trial methylprednisolone was given at a bolus dose of 30 mg/kg at 24 hours before laser injury to the retina. Continuous infusion of methylprednisolone (5.4 mg/kg/hr) was started one hour after the bolus injection and stopped at 4 days afterward. Preliminary evaluation of the efficacy of the methylprednisolone in laser induced retinal lesion was assessed at 3 days and 10 days after retinal injury using clinical features.

B. Clinical Cases

Patients suffering from laser-induced retinal injury were followed clinically: visual acuity, Amsler grid, and fundus examination. Fundus photography and fluorescein angiography were recorded on a regular basis until the clinical features stabilized.
III. Results

A. Clinical features of Grades II, and III retinal lesions in normal monkeys without medication (Fig 1 and 2). Figures 1, and 2 are fundus appearances of Grades II, and III lesions at 3 (Fig 1) and 10 days (Fig 2) after laser injury. At 3 days after laser injury, Grade II lesions showed grayish spots on fundus picture. Grade III lesions showed larger spots each with a round whitish center and grayish ring at the periphery. At 10 days after laser injury, Grade II lesions showed comparable grayish appearance as on day 3. The Grade III lesion showed a diminished whitish center. Figures 3 and 4 are fluorescein angiograms of Grades II, and III lesions taken at 3 (Fig 3) and 10 days (Fig 4) after injury. Both Grades II and III lesions at 3 days and 10 days showed severe leakage.

B. Histopathologic features of Grades II, and III lesions in normal monkeys without medication: Figure 5 showed sections of Grade II retinal lesions from 3 days up to 20 days after laser treatment. At 3 days (A) after laser injury, choriocapillaries showed partial narrowing. Retinal pigment epithelium exhibited total necrosis with macrophages at the edge of the lesion. The outer segments showed coagulative necrosis and the inner segment showed focal densification. The outer nuclear layer had total coagulative necrosis with pyknotic nuclei. The outer plexiform layer were vacuolated. The inner nuclear layers and inner retina were unremarkable. At 10 days (B) after the injury, the choriocapillaries showed partial recovery. The RPE exhibited regeneration (single layer). A few pigment-ladened macrophages in the subretinal space were noted. Outer segments and inner segments were totally absent. There was total loss of outer nuclear layer at the center of the lesion. The inner retina appeared to be unremarkable. At 20 days after injury, the choriocapillaries reopened. RPE showed proliferative reaction focally. There was a decrease in the number of macrophages but they could still be seen in the subretinal space. At the center of the lesions there was total absence
of outer nuclear layer and pyknotic nuclei were still noted at the periphery of the lesion. Edema of the outer plexiform layer subsided. The inner layer appeared unremarkable.

Figure 9 showed Grade III retinal lesions from 3 days up to 20 days after laser treatment. At 3 days (A) after the injury, the width of the lesion appeared to be larger than Grade II lesions. The choriocapillaries were occluded. RPE was necrotic. Macrophages were noted at the edge of the lesion. Both outer and inner segments showed photocoagulation and total necrosis. There was total loss of photoreceptor cells at the center of the lesion and pyknotic nuclei were noted at the periphery. The inner nuclear layer showed pyknotic nuclei with coagulated necrosis and edema. At 10 days (B) after laser injury, the choriocapillaries remained occluded. There was active RPE proliferation. Pigment-laden macrophages were noted. There was total loss of outer nuclear layer at the center of the lesion. Macrophagic cells were noted in the inner retina. The inner nuclear layer showed focal necrosis and scattered pyknotic nuclei were noted. At 20 days (C) after the injury the choriocapillaries were partially open but appeared to be narrowed. Multi-layered RPE cells were noted with macrophages in the subretinal space. There was total loss of photoreceptor cells and pigment-laden macrophages were noted. In the inner retina, vacuolization and edema were noted. There was loss of inner nuclei and pyknotic nuclei. At the center of the lesion, there was total loss of inner nuclear layers in addition to the total loss of photoreceptor cells.

C. Morphometry of Grades II and III lesions. Figure 7 showed that the outer nuclear layer gap and the retinal pigment epithelial depigmentation in Grade II lesions were dependent on the time after injury. There was a sharp decrease from 3 days to 10 days and remained unchanged from 10 to 20 days after laser injury. Similar time-dependent relationship existed in the Grade III lesions.
Figure 8 showed the relationship between the laser energy and the outer nuclear gap, and the retinal pigment epithelium depigmentation measured at 10 days after injury. There was linear relationship between the energy applied and the parameters measured: namely the outer nuclear layer gap and the retinal pigment epithelial depigmentation.

D. Drug delivery. One monkey was used in testing each of the drug delivery systems. The cast system was bound to be unsatisfactory because the animal disrupted the implanted catheter after 8 hours of infusion. The commercially available tethering system was satisfactory and was used continuously for 5 days with no problem.

E. Efficacy of methylprednisolone on laser induced retinal lesions. From fundus photographs and fluorescein angiograms at 3 days and 10 days after injury, we were able to detect ameliorative effects with methylprednisolone. Figure 9 showed the fundus appearances of Grade II and III retinal lesions in the methylprednisolone-treated monkey at 3 days (A) and 10 days (B) after laser injury. In contrast to the control (Fig 1), in the methylprednisolone-treated retina (Fig 9A) at 3 days Grade II lesions (a) were faint and could hardly be seen, and the Grade III lesions (b) were smaller and showed no whitish center but a mild grayish spot (see Fig 5). At 10 days after laser injury with methylprednisolone treatment (Fig 9B), Grade II lesions (a) could not be easily identified. The Grade III lesions (b) showed no whitish center in contrast to control (Fig 2). Hence, the fundus pictures showed less severe damage to the retina in the treated group. Figure 10 showed the fluorescein angiography of the methylprednisolone-treated laser lesions. In the methylprednisolone-treated lesions, at 3 days (Fig 10A) after laser treatment, there was severe leakage in Grade III lesions (b) and they were comparable to those of the control. Minimum leakage was seen in the Grade II lesions (a). But at 10 days (Fig 10B) after laser injury there was no leakage in
both Grades II (a) and III (b) lesions in the methylprednisolone-treated retina but a subtle window effect was noted. Therefore, from the clinical appearance, methylprednisolone treatment appears to have beneficial effects. Histopathologic and morphometric studies are underway.

D. Clinical cases

Case 1. Patient #1 was a 29-year-old female graduate student. Her left eye was injured by YAG laser. The exact energy level of the laser beam to the retina was not known. She claimed to wear green goggles at the time of the accident. She was examined one week after the accident. Her visual acuity was 20/60 on the affected eye, with blurred and distorted vision and central scotoma. Fundus examination showed a paracentral retinal burn with macula edema and sub-RPE hemorrhage. Fluorescein angiography showed a central area of hypofluorescence corresponding to the area of hemorrhage. Five weeks later, her visual acuity was still 20/50, with blurred and distorted vision and central scotoma. Blood spread from sub-RPE region to the subretinal space and organized. One month later, her visual acuity improved to 20/30 with distorted vision and central scotoma. However, a lamella hole in the fovea developed (Fig 11).

Case 2. Patient #2 was a 24-year-old male graduate student. He was injured by YAG laser. The energy of the incident light into the retina was not known. He also claimed to wear "argon laser goggles". Immediately after the accident his visual acuity was 20/200, with distorted and blurred vision. One month later, his visual acuity improved to 20/40 with central scotoma and necrotic retina. Another month later, his visual acuity was 20/50, with distorted and blurred vision and central scotoma. At that time, a macular hole was noted with shallow serous macular detachment (Fig 12). One month later, his vision improved to 20/30. Subsequently, fundus examination showed no further changes.
IV. Conclusion

During this reporting period, we were able to document the clinical and histopathological changes of 2 grades of retinal lesions induced by laser. The two quantitative parameters proposed namely, the outer nuclear layer gap and the width of retinal pigment epithelial depigmentation appeared to be adequate for the evaluation of drug efficacy. The commercially available tethering system for continuous infusion of corticosteroids into primates was tested and found to be satisfactory. Preliminary findings from our studies with high doses of methylprednisolone suggested that the drug is effective in ameliorating laser-induced retinal lesions when it is given before injury and continued up to three days after injury. This observation was supported by the clinical and preliminary histopathological features. However, more animal experiments and morphometric data are still in progress to provide statistical analysis for confirmation of these initial observations.

In contrast to earlier clinical use and experimental models, the dose we used in this last set of experiments using methylprednisolone is very high. We followed the drug regimen as reported in the clinical trial of methylprednisolone in acute spinal cord injury in human. A bolus injection of 30 mg/kg was given to the animal at 24 hours before laser injury and continuous infusion of methylprednisolone at a rate of 5.4 mg/kg was started at 1 hour after the bolus injection until 3 days after laser injury. Our preliminary findings that this drug dose scheduled is effective in ameliorating laser-induced retinal lesions are very encouraging.

To conclude, our method for continuous drug delivery to primates, and evaluation of retinal damage are satisfactory. High doses and short term continuous infusion of methylprednisolone appears to have an effect on laser-induced retinal lesions. However, whether it has therapeutic usage must be
determined by varying the time of administration of methylprednisolone after laser injury.

Two patients who were accidentally injured by YAG laser were studied clinically. Both claimed to wear protective goggles but with the wrong cutoff spectrum and both were graduate students. It is apparent that the education for laser safety is insufficient. In both cases, subretinal hemorrhage was observed, and followed similar clinical courses with initial dramatic decrease in visual acuity, gradual improvement of visual acuity, scar formation, and macular hole development. Therefore, hemorrhage and complications after laser injury should be of primary concern in developing strategies in the treatment of laser-induced retinal injury.
Diagram 1. Measurements of outer nuclear layer (ONL) gap (A) and the width of the depigmented RPE (B) in laser-induced retinal lesion (Grade III).
Diagram 2. Continuous intravenous infusion system in primates.  
(A) Implantation of catheter.  (B) Fixation with adhesive tapes.  (C) Connection of steel conduit and infusion tubes.  (D) Positioning of the orthoplastic cast.  (E) Fixation of the orthoplastic cast.
Diagram 3. Commercially available tethering system with jacket and swivel.
Figure 1. Fundus pictures of Grades II and III lesions at 3 days after induced injury. Grade II (b) lesions showed grayish spot on fundus picture while Grade III (a) lesions showed larger lesions each with a round whitish center and a grayish ring.
Figure 2. Fundus pictures of Grades II and III lesions at 10 days after laser injury. Grade II (b) lesions showed similar grayish appearance as that of the 3 day lesion while Grade III (a) lesions showed a small whitish center and grayish ring. The size remained the same as that at 3 days.
Figure 3. Fluorescein angiograms of Grades II and III lesions at 3 days. Both Grades II (a) and III (b) lesions showed severe leakage.
Figure 4. Fluorescein angiogram of Grades II and III lesions at 10 days after injury. Similar to day 3, both Grades II (a) and III (b) lesions showed severe leakage.
Figure 5. Histopathologic features of Grade II lesions at (A) 3, (B) 10, and (C) 20 days after laser injury. For explanation see text.
Figure 6. Histopathologic features of Grade III lesions at (A) 3, (B) 10, and (C) 20 days after laser injury. For explanation, see text.
Figure 7. Time dependent relationship of the outer nuclear gap and the retinal pigment epithelial depigmented gaps in Grade II lesions.
Figure 8. Energy dependent relationship of the outer nuclear layer gap and the retinal pigment epithelial gap.
Figure 9. Fundus picture of Grades II and III retinal lesions at 3, and 10 days after laser injury in methylprednisolone-treated monkey. (A) Three days after laser injury. Grade II lesions (b) were very difficult to identify. Grade III lesions (a) showed no whitish center as compared to the control lesions, but each with a grayish spot. The spot size was smaller than the control (see Fig. 5). (B) At 10 days after laser injury. Grade II lesions (b) could hardly be identified. Grade III lesions (a) showed no whitish center but a grayish spot. The spot size was smaller than the control.
Figure 10. Fluorescein angiogram of the methylprednisolone-treated laser lesions. (A) At 3 days. Grade III (a) lesions showed severe leakage and appeared to be comparable to those of the control. Minimum leakage was noted for Grade II lesions (b). (B) At 10 days. There was no leakage in both Grades II (b) and III (a) lesions but subtle window effect for the Grade III lesion.
Figure 11. Fundus picture of patient #1 at 3 months after the initial injury. A lamella hole in the fovea was noted.
Figure 12. Fundus picture of patient #2 at 3 months after laser injury.
References


