Argon laser phototherapy of Pseudomonas corneal ulcers

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A new technique with the use of argon laser irradiation of Pseudomonas organisms stained with fluorescein-tagged antibody has proved beneficial in the treatment of these particular corneal ulcers in rabbits. This specific phototherapy introduces a concept that may have significance in future clinical studies.

Key words: argon laser, Pseudomonas aeruginosa, antigen–fluorescein antibody complex, differential staining.

The advent of the laser in 1960 made an extremely intense, highly directional, monochromatic beam of light that operates in the visible portion of the spectrum accessible to the world for the first time. Ophthalmic investigators quickly became aware of the enormous potential of the laser as a photocoagulation tool. The high predictability of absorption of laser radiation by the ocular structures, the precise focusing capabilities of the monochromatic coherent light beam, and the high photon energy density were all attractive facets of the laser principle. The first actual laser trials in 1961 and 1963 affirmed the efficacy of laser photocoagulation. The lasers available for photocoagulation have increased during the past several years (ruby, argon, krypton, neodymium, carbon dioxide) with the result that various monochromatic beams can produce selective histopathologic alterations of ocular tissue. Retinal tears, retinoschisis, peripheral retinal degeneration, various vascular abnormalities such as microaneurysms, neovascularization, and angiomata, chorioretinitis, maculopathies, intraocular tumors, and other abnormalities have been treated with laser photocoagulation. Although the laser has been extensively employed as a retinal photocoagulation device, it has not yet been used as a means of combatting corneal infections. This paper is concerned with studies conducted to determine the in vivo efficacy of argon laser radiation in the treatment of Pseudomonas aeruginosa (pyocyanous) corneal ulcers stained with a fluorescein antibody.

Pseudomonas aeruginosa is one of the most destructive of all ocular infectious agents. In the majority of instances, the course of an untreated ulcer leads to widespread corneal suppuration and perforation within a few days. The pseudomonas...
organism is refractory to therapy with the usual antibiotics. Prior to the advent of polymyxin it was assumed that the poor results of therapy were due to the high innate resistance of the Pseudomonas organism to antibiotics and chemotherapeutic agents. Wiggins\textsuperscript{7} demonstrated that polymyxin B is a much more effective antibiotic agent against \textit{Pseudomonas aeruginosa} than are chlorotetracycline or streptomycin. He also demonstrated arrest and cure of Pseudomonas corneal ulcers when polymyxin B therapy was instituted 6, 12, and 18 hours after inoculation of the rabbit cornea with organisms. If used 24 hours after inoculation, the treated eyes showed progression of the ulcer even though they did not perforate. It has also been shown that once the organism is firmly established, therapy is, at best, globe saving, but never sight saving. Experiments in which therapy was delayed 48 hours after inoculation showed only slight effect in controlling the infection of Pseudomonas even though large antibiotic concentrations were administered.

The continuous drip technique reported by Hessburg and associates\textsuperscript{8}\textsuperscript{19} employing subpalpebral tubes inserted into the cul-de-sac after intubation of the upper lid (6 to 8 drops per minute of colistin sulfate [0.05 per cent] and acetyl sulfanilamide [0.05 per cent] in normal saline) appears to be one of the more effective techniques at present for combatting Pseudomonas ulcers. This continuous subpalpebral lavage is maintained for 14 days with subsequent antibiotic treatment for a minimum of six weeks. The course of a Pseudomonas ulcer under such treatment is not one of dramatic improvement. Extreme tissue destruction precludes any rapid change for the better. Another quite effective technique for sterilizing Pseudomonas human corneal ulcers with the episcleral perlimbal injections of gentamicin (Allen, Furgiele, Golden\textsuperscript{9}).

In this research, Pseudomonas (attacking the cornea) was stained with a fluorescent antibody (Pseudomonas specific) in order to induce fluorescent absorption of photon energy emitted by an argon laser. Fluorescence is defined as the absorption of photon energy followed by a subsequent emission of photon energy at a longer and less energetic wavelength usually within $10^{-7}$ seconds, where the energy difference goes to excite the separate atoms that make up the fluorescent molecule, or fluor. The extent to which a particular wavelength of light stimulates the fluorescence or absorption of a fluor under constant conditions is dependent only upon the degree to which photons of that wavelength are absorbed by the fluor. Thus, although a photon of ultraviolet light at 3,600 Å has greater energy than a photon at 4,880 Å (the wavelength of the argon laser beam), the latter is more effective for inducing fluorescent absorption in fluorescein because, as can be seen in the absorption curve (Fig. 1), it is precisely the wavelength most likely to be absorbed (absorption peak). Thus, we have at our disposal a fluorescent stain that will absorb over 90 per cent of the entering photon energy at 4,880 Å. The uniqueness of the argon gas laser is that

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fluorescein_absorption.png}
\caption{Curve showing the absorption peak of fluorescein which corresponds to the argon laser wavelength, 4,880 Å.}
\end{figure}
it can supply the investigator with a virtually pure source of directional, highly intense coherent light at precisely the 4,880 Å wavelength.

At this point, the efficacy of maximizing photon energy absorption by first staining with fluorescein and subsequently irradiating with argon laser radiation at the 4,880 Å wavelength is apparent.

The complication now present is the fact that the investigator may desire to have certain areas differentially stained while leaving other areas unstained and unaffected. To stain a corneal ulcer with only fluorescein would result in the simultaneous staining of unaffected adjoining areas of the ulcer and, even though the laser is highly directional, these adjoining areas would absorb some of the intense argon laser radiation from reflection, refraction, and scattering effects. It is highly desirable to have these uninfected corneal surfaces totally unstained and thus non-absorptive to the 4,880 Å wavelength. It is possible to differentially stain an antigen with a fluorescent group like fluorescein by introducing an intermediate antibody which is specific for the infecting antigen (Pseudomonas) and which is also tagged with the fluorescent group (called the fluorescent antibody technique as demonstrated by Coons and colleagues 10). The direct staining of antigens with a fluorescent antibody is shown schematically in Fig. 2. A fluorescein-labeled antibody (in this work it was Pseudomonas specific) is applied to an antigen (Pseudomonas) and allowed to conjugate for an interval of time. Excess antibody is then rinsed away.

After the conditions for the staining of an antigen have been established and its staining has been accomplished, it is then necessary to prove that the observed staining is specific. That is, does the observed result represent a reaction between antigen and its specific antibody? A proof of such specificity is that uninfected tissue or uninvolved cells will not be stained. Such in vivo proof has been observed in rabbit corneal Pseudomonas ulcers where uninfected corneal tissue was not stained. The proof was made when the stained rabbit ulcers were irradiated with the 4,880 Å argon laser beam, during which time the uninfected corneal areas displayed no observable fluorescence and thus little absorption of energy. The laser beam traversed the uninfected corneal tissue without loss of energy and, hence, without affecting this transparent tissue. These experimental results suggest that the 4,880 Å wavelength might differentially destroy the stained highly absorbent organisms without damage or impairment to adjacent transparent corneal tissue. Histologic examination (3 weeks after irradiation) of the treated rabbit eyes has shown no adverse effects from the irradiation procedure.

Materials and methods

Ten different strains of Pseudomonas, isolated from human infections, served as the test organisms in this study. The different strains of Pseudomonas came from eye (two strains), sputum (two strains), urine (two strains), stool (two strains), heart (one strain), and blood (one strain). In vitro determinations were made on these ten strains to determine the power levels at which the organisms were sensitive to argon laser radiation. On clear agar (employed to minimize

![Diagram of the reaction](https://example.com/diagram.png)

Fig. 2. Schematic representation of the direct staining of an antigen with a fluorescent antibody.
absorption effects by the agar itself), 1 to 2 mm. diameter spots of Pseudomonas were cultured and then conjugated for eight minutes with 6 μc of fluorescein antibody specific for Pseudomonas deposited by means of a micropipette. Spots, rather than streaks, of organisms separated a substantial distance from one another were irradiated to avoid the possibility, upon transference to another agar plate by means of a platinum loop, of picking up adjoining organisms which had not been irradiated. The organisms were exposed for various intervals of time and also at various intensities of laser radiation by the method illustrated in Fig. 3. At 400 mw. per square millimeter, an exposure of nine minutes was required to destroy the organisms in vitro. At 600 mw. per square millimeter, destruction was accomplished by an exposure of seven minutes and at 850 mw. per square millimeter, an exposure of two minutes was adequate. The destruction of unstained colonies required a considerably longer exposure at each level of intensity. After irradiation the colonies appeared morphologically unchanged. No charring effects were evident suggesting that the increase in temperature was adequate for killing the organisms and yet without any apparent effect on neighboring nonabsorbent materials. Measurements with a chromel-alumel thermocouple confirmed the fact that a sufficient rise in temperature was induced by the argon laser radiation to be responsible for the death of the Pseudomonas.

In vitro controls were run where the organisms were only conjugated with the fluorescein antibody for the eight-minute intervals (not irradiated) and then transferred to another agar plate. These controls yielded positive cultures—thus proving that the fluorescein antibody alone has no marked inhibitory effect upon the Pseudomonas organisms. All test colonies, just prior to irradiation, were also cultured to make certain that a viable colony existed.

Chinchilla and albino rabbit corneas were inoculated with the various strains of Pseudomonas by creating 2 to 3 mm. tracks with a hypodermic needle that penetrated into the stroma. Both eyes were inoculated similarly with the expectation that one eye would serve as a control. Although 17 rabbits were inoculated, only 13 cases were actually treated as subsequently reported in this paper. The unreported cases involved ulcers that either followed benign courses or proceeded at different rates in fellow eyes.

It is well to remember that Pseudomonas aeruginosa is, in some instances, a saprophyte which possesses little or no virulence in the eye. This has been observed during this work by the experimental production in animals of Pseudomonas corneal ulcers which have relatively benign courses even when untreated. Fulminating corneal ulcers can be produced experimentally by the use of virulent strains. Only ulcers created by these virulent strains were treated by the argon laser technique; a parameter that lends added significance to any positive experimental results.

Having inoculated both eyes, the organisms were allowed to incubate for various periods of time. Rabbits were then anesthesized with 1 ml. of diabutal injected intravenously. The ulcers in both eyes were conjugated with the fluorescein antibody for eight minutes. Prior to conjugation with the fluorescein antibody, the control ulcer and the experimental ulcer was wiped lightly with a cotton applicator to remove the excess purulent discharge. Such white material would only serve to reflect or interfere with the argon laser photon energy absorption by the antigen–fluorescein antibody complex.

The removal of the purulent discharge appar-
Table I.

<table>
<thead>
<tr>
<th>Case</th>
<th>Ulcer size (time of treatment) (mm.)</th>
<th>Ulcer size (time of treatment) (hr.)</th>
<th>Source of strain</th>
<th>Treatment</th>
<th>Culture of treated eye*</th>
<th>Culture of control eye after staining</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
<td></td>
<td></td>
<td>Amount (mg.)</td>
<td>Time (min.)</td>
<td>Before irradiation</td>
</tr>
<tr>
<td>1</td>
<td>2 3</td>
<td>25</td>
<td>Urine</td>
<td>700 2</td>
<td>+ -</td>
<td>+</td>
<td>x</td>
</tr>
<tr>
<td>2</td>
<td>4 4</td>
<td>26</td>
<td>Sputum</td>
<td>800 2</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>3</td>
<td>3 3</td>
<td>25</td>
<td>Eye</td>
<td>800 2</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>4</td>
<td>4 4</td>
<td>27</td>
<td>Eye</td>
<td>850 2</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>5</td>
<td>4 5</td>
<td>29</td>
<td>Heart</td>
<td>850 2.5</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>6</td>
<td>6 8</td>
<td>43</td>
<td>Blood</td>
<td>850 3</td>
<td>+ -</td>
<td>+</td>
<td>XX</td>
</tr>
<tr>
<td>7</td>
<td>8 9</td>
<td>49</td>
<td>Urine</td>
<td>850 3.5</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>8</td>
<td>8 9</td>
<td>45</td>
<td>Eye</td>
<td>850 2.5</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>9</td>
<td>9 9</td>
<td>51</td>
<td>Sputum</td>
<td>850-900 2.5</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>10</td>
<td>10 10</td>
<td>50</td>
<td>Stool</td>
<td>850 3</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>11</td>
<td>10 11</td>
<td>58</td>
<td>Urine</td>
<td>850 3.5</td>
<td>+ -</td>
<td>+</td>
<td>X</td>
</tr>
<tr>
<td>12</td>
<td>11 13</td>
<td>59</td>
<td>Heart</td>
<td>850 4</td>
<td>+ -</td>
<td>+</td>
<td>XXX</td>
</tr>
<tr>
<td>13</td>
<td>13 14</td>
<td>61</td>
<td>Stool</td>
<td>850 4</td>
<td>+ -</td>
<td>+</td>
<td>XXX</td>
</tr>
</tbody>
</table>

*In the above chart + refers to a viable colony, - to a nonviable colony.
†X = Central infiltration; healed with slight scarring.
‡XX = Dense central infiltration; healed with dense scar.
§XXX = More than one half of cornea infiltrated; healed with dense scar.
‖XXXX = Whole cornea infiltrated; healed with completely vascularized scar.

This is graded according to the method employed by Robert L. Wiggins.²
ently did not inhibit or affect the control ulcers because the control eyes consistently followed typical destructive courses. After conjugating the ulcer with the fluorescein antibody, small amounts of saline solution were dropped on the uninfected areas of the cornea to assure that any excessive stain was rinsed away. The rinsing procedure eliminated the possibility that uninfected areas would absorb significant photon energy. Cultures of both infected eyes were conducted after the staining procedure, but prior to laser irradiation, to affirm that the experimental and control ulcers were viable. All such cultures produced pure growths of Pseudomonas. Similar cultures of the irradiated eyes were made after irradiation to determine the effect of laser radiation on the viability of irradiated organisms and the results of these cultures are reported in Table I.

Having treated both eyes in the same manner, the larger and more rapidly progressive ulcer was chosen for irradiation. The sizes of the treated and control ulcers are reported in Table I. The attempt to irradiate the larger ulcers was made since such ulcers were clinically prevalent.

Laser irradiation of the cornea was performed by means of an articulated arm delivery system described in detail elsewhere.17 Basically, this device guides the output of the laser through a series of hollow, jointed arms that terminate in a small handle. The handle is approximately the size of a fountain pen and can be moved easily in any direction by the surgeon; a delivery system that provides full trimensional mobility. The system consists of an alternating series of six hollow tubular sections and six hollow blocks as illustrated in Fig. 4. The addition of a 40 diopter lens to the tip of the handle focuses the light to a point-sized image 25 mm. away. Corneal irradiations were conducted 45 to 50 mm. from the tip in order to create a divergent, low power density beam at the treatment area. The laser beam was produced by a Coherent Radiation Laboratories (Type 53) argon gas laser (Palo Alto, Calif.).

The beam was directed tangentially across the cornea and moved slowly through the ulcer. The period of irradiation was divided into 50 to 60 second intervals. For example, an ulcer may have been irradiated for three 50 to 60 second intervals totaling three minutes as recorded in Table I which summarizes the results.

Results

The results of the 13 cases studied are summarized in Table I. Of the 13 control eyes, seven perforated and the other six developed completely vascularized corneas. None of the treated eyes were lost due to corneal perforation and the progress of corneal infections was arrested in ten of the 13 cases. The results on the table show that ulcers of shorter duration are more susceptible to the laser treatment. Of these younger ulcers (51 hours and less) which were arrested and subsequently healed, six resulted in slight scarring (Cases 1-5, and 8). Three other cases (Cases 6, 7, and 9, which were 43, 49, and 51 hours old, respectively) healed with dense scarring and slight vascularization in Cases 7 and 9. In Case 10, where well over one half of the cornea was infected, the results were healing with a dense scar and slight vascularization. All of these cases yielded pure cultures of Pseudomonas prior to irradiation (after the staining procedure) and cultures of eyes after irradiation showed no viability.

Cases 11 (11 mm. in diameter and 58 hours old) and 12 (13 mm. in diameter and 59 hours old) were only moderately affected by irradiation since both ulcers temporarily ceased progression for periods of 30 (Case 11) and 34 hours (Case 12). In Case 11, a positive culture of Pseudomonas was obtained not only before irradiation, which is normal, but after irradiation, indicating that there were still viable organisms present. Case 13 (61 hours old) showed no temporary cessation of growth and yielded positive cultures of Pseudomonas both before and after irradiation. Case 13, the largest ulcer irradiated, resulted in a totally vascularized cornea.

In the eyes of the ten rabbits treated and cured with the laser technique, no detectable physiologic or histopathologic changes in the other ocular tissues were observed. When tested for moving-object detection and general perception, vision in these ten rabbits seemed unimpaired.

Comment

Both control and irradiated eyes were processed in the same manner with respect to the removal of purulent discharge, fluorescent staining, and saline rinsing. All control ulcers followed typical destructive courses indicating that the staining procedure used on the eyes prior to irradiation
had no marked inhibitory effects upon the Pseudomonas. Therefore, the positive responses of the irradiated ulcers can be attributed only to the laser treatment because the other factors (removal of excess purulent material, fluorescein antibody staining, and the rinsing of stain from uninvolved areas) appears to have had no inhibitory effects on the Pseudomonas ulcers, as determined experimentally.

Case 11, which gave a positive culture of Pseudomonas before and after irradiation, was one of the two ulcers which exhibited temporary cessation. This temporary inhibition of the ulcers in Cases 11 and 12 might be due to a reinfection after the laser irradiation from a small number of organisms still remaining which re-establish themselves and commence normal growth.

If any treatment of Pseudomonas aeruginosa ulcers is to be effective, prompt clinical recognition of the lesion is essential. Cultures must be confirmatory and not diagnostic, for an early therapy must be instituted if any useful vision is to be maintained. Efficacy of therapy must be immediate since the course of Pseudomonas is so rapid and devastating. The laser technique offers such a therapy.

Any therapy must be not only immediate in its effect, but also gentle and safe. Argon laser radiation in this study induced no significant adverse side effects as confirmed by histologic studies. The ulcers and the adjoining areas appear after irradiation much as they did prior to irradiation, indicating that no charring effects are producing the results, but rather a gentle elevation in temperature of the infected areas.
as confirmed in vivo by chromel-alumel thermocouple studies.

These in vivo chromel-alumel thermocouple studies confirmed the efficacy of fluorescein antibody staining, as unstained ulcers rose slightly in temperature upon steady irradiation. These same ulcers, when conjugated with the fluorescein antibody (all other parameters such as thermocouple depth into the ulcer and laser distance being the same as with the unstained ulcer) exhibited immediate and sharp rises in temperature (over 90° C). The thermocouple employed was extended through a hypodermic needle so that the active junction could be suspended by a micromanipulator into the ulcer itself, rather than merely on the surface. By so doing it was possible to measure the temperature in the infected stroma.

The effectiveness and the exceptional rapidity of action of the laser technique are emphasized by the fact that only a few minutes were required to eliminate the infection. As predicted by these investigators, the tangential irradiation of Pseudomonas ulcers (stained with a fluorescein antibody) by an argon laser beam considerably facilitated therapy. Hence, the technique should be considered as an addition or an alternative to the intramuscular and topical routes of administration in the treatment of ocular Pseudomonas infections. The efficacy of argon laser therapy in Pseudomonas suggests further investigation into infections of the cornea caused by such pathogens as Candida albicans, Aspergillus, Herpes simplex, Pneumococcus, Staphylococcus, and Streptococcus.

REFERENCES
9. Personal communication.