Anterior Stromal Puncture with the Nd:YAG Laser

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A simple and consistent puncture of the anterior stroma was created with the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser in a rabbit model. Varying energy levels were tested, and settings at 2.0 mJ produced well-formed anterior stromal breaks. The laser shock wave disrupted Descemet's membrane in only 1 of 50 animals. The wound healed normally in vivo over a 3-month period. Compared to needle puncture, the laser puncture created less stromal scarring. Initial human studies also showed that energy levels from 1.5–2.5 mJ were sufficient to create local breaks in Bowman's layer. Further human studies will be needed to determine if Nd:YAG laser puncture offers any advantages over current techniques for stromal puncture in the treatment of traumatic recurrent corneal erosions.


Stromal puncture has been advocated as a useful technique to treat difficult cases of traumatic recurrent corneal erosions (RCE). Previous authors have used microdiathermy1–3 and anterior stromal puncture with a 20- or 27-gauge needle4–6 to breach Bowman's layer to provide a stronger adhesion of epithelium to the underlying stroma. Both of these procedures produce stromal scarring in the areas of application, which makes the physician wary of using the technique for central erosions. A simple, refined disruption of the anterior stroma was achieved with the neodymium:yttrium-aluminum-garnet (Nd:YAG) laser in a rabbit model.

Materials and Methods

All investigations involving animals reported in this study conform to the ARVO Resolution on the Use of Animals in Research.

Determination of Proper Power Setting

The laser used in this study was a Coherent Nd:YAG laser, model 9900 (Palo Alto, CA). Enucleated rabbit eyes (n = 28), denuded of epithelium with the use of n-heptanol (Sigma Chemical, St. Louis, MO),5 were treated with multiple single pulses at 1.0, 1.5, 2.0, 2.5, 3.0, and 6.0 mJ without the use of a contact lens, to determine the proper energy levels to produce anterior stromal breaks. Fluorescein sodium 0.25% topical anesthetic solution (Fluress; Barnes-Hind, Sunnyvale, CA) was applied to the eye, and the cobalt blue filter used. The helium–neon beam could then be focused easily on the basement membrane. Excised corneas were placed in Trump's fixative and prepared for routine light and transmission electron microscopy.

In Vivo Studies

New Zealand albino rabbits, weighing 2–3 kg, were anesthetized with intramuscular ketamine hydrochloride (Parke-Davis, Morris Plains, NJ) (200 mg) and chlorpromazine hydrochloride (Goldline Laboratories, Ft. Lauderdale, FL) (25 mg). Topical 0.5% proparacaine hydrochloride (ER Squibb, Princeton, NJ) was instilled. Bilateral central 3.0-mm n-heptanol cornea wounds were made. One eye was treated at 2.0 mJ with multiple (100) single pulses as described above (n = 20). Animals were examined at 2.0 mJ with multiple (100) single pulses as described above (n = 20). Animals were examined with 0.25% fluorescein dye daily for the first 2–3 days to monitor times for reepithelialization. Erythromycin ointment was applied to both eyes once daily for 2–3 days until the central defect had closed. Animals were sacrificed at 2, 7, 14, 28, and 84 days (n = 2–5 per time point), and the corneas prepared for routine light and electron microscopy. In a separate series of experiments, comparative in vivo healing was assessed by treating one eye with 25 stromal punctures with a bent 26-gauge needle and the other eye with 50 single pulses at 2.0 mJ. The rabbits were examined with the slit lamp on days 2, 7, 14, 28, 42, and 70 (n = 4) and the amount of stromal scarring noted.

Human Studies

After signed consent, patients (n = 5) who were scheduled to undergo penetrating keratoplasty for
bullous keratopathy had the central 3 mm of corneal epithelium debrided at the slit lamp. Approximately 50 Nd:YAG pulses at 1.5, 2.0, 2.5, or 3.0 mJ, respectively, were placed. One hr later, the corneal button was excised in the operating room and placed in Trump's fixative and processed for routine light and transmission electron microscopy.

Results

Power Settings

Reproducible disruptions of the anterior corneal stroma were produced with the Nd:YAG laser. At 1.0 mJ, the basement membrane was focally disrupted with minimal stromal involvement (Fig. 1A). At 2.0 mJ, an anterior stromal break averaging 2.1 μm (range 0.5–4.8 μm) occurred. Focal collagen disruption was seen at the edges of the break but not deeper in the tissue (Fig. 1B). At 2.5 and 3.0 mJ, larger and deeper breaks occurred. Again, collagen disruption was seen at the edges of the break. Larger breaks with widespread collagen disruption were seen at 4.0 and 6.0 mJ.

In Vivo Studies

At 2 days, an epithelial plug was present in the anterior stromal cleft (Fig. 2). Basal epithelium protruded through the break in the basement membrane. Disrupted collagen bundles surrounded the epithelial plug. The stroma underneath the defect appeared relatively unaffected. One sample showed a break in Descemet's membrane (1 of 50 = 2% incidence) underlying the anterior stromal break. An accompanying proliferation of endothelial cells was seen over Descemet's membrane in this region. At 1 week, the epithelial plug began to retract and fit snugly in a sharply demarcated anterior stromal break. Collagen

Fig. 1. Varying Nd:YAG laser energy levels to produce stromal wounds in the rabbit cornea. (A) 1.0 mJ. Focal disruption of basement membrane with minimal stromal involvement (×6000). (B) 2.0 mJ. Well-defined anterior stromal break with no collagen disruption below puncture (×1500). Inset: magnification (×400) of (B).

Fig. 2. Two-day in vivo rabbit cornea. Epithelial plug fills well-defined anterior stromal break. Undisrupted collagen fibers are seen below anterior stromal wound (×1500).
Fig. 3. One-week in vivo rabbit cornea. Epithelial plug fits snugly in anterior stromal break. Plug is beginning to retract (×1500).

Fig. 4. Two-week in vivo rabbit cornea. Epithelial plug has retracted. Activated fibroblasts are seen under normal appearing stratified epithelium (×1000).

Fig. 5. Four-week in vivo rabbit cornea. Disrupted collagen (arrowhead) seen immediately below epithelial layer. Basement membrane formation (arrow) is incomplete in this section (×24,000).

bundes surrounding the anterior break were disrupted while those posterior appeared intact (Fig. 3). At 2 weeks, the plug had retracted. Activated fibroblasts were present under the intact epithelium in the superficial stroma and aligned themselves vertically along the laser incision (Fig. 4). In a few sections, rare inflammatory cells occasionally were seen in this location. Basement membrane reformation was incomplete at this stage. At 4 weeks, the epithelium had completely retracted, and the basement membrane was almost completely intact, with some discontinuity of the basement membrane seen in several locations. Disrupted collagen bundles were seen immediately underlying the epithelium (Fig. 5). At 3 months, the overlying epithelium appeared normal and the anterior stromal wound had healed (Fig. 6).

All treated and untreated corneal defects healed by 48 hr, and no recurrent erosions were produced by the laser procedure. Minimal scarring was seen in the laser-treated eyes at all time points. In contrast, distinct triangular stromal scars were seen in all needle punctured eyes for the period of observation (Fig. 7).

Human corneas treated at 1.5 mJ had defects which partially penetrated Bowman’s layer. In one specimen, at 2.0–2.5 mJ, Bowman’s layer was precisely cut (1.66 μm in depth) (Fig. 8). A larger anterior stromal cut with more collagen disruption occurred after a 3.0-mJ treatment.

Discussion

Tight basal epithelial attachments are established by means of basement membrane complexes that consist of hemidesmosomes, basement membrane, and anchoring fibrils. RCE is a common clinical en-
Both microdiathermy and needle stromal puncture produce stromal scarring, which makes these procedures less suitable for central corneal erosions. It is quite difficult to achieve uniform depths of puncture with a needle. Deep or oblique punctures will create larger triangle-shaped scars. In addition, the microdiathermy unit itself may not be readily available to physicians in a typical office setting. The Nd:YAG laser produced reproducible shallow incision depths. Compared to the needle puncture, it caused only minimal stromal scarring, which was an encouraging result. Rare inflammatory cells were seen in only a few specimens. Theoretically, one could produce multiple breaks in Bowman’s layer sufficient to allow normal basement membrane complexes to form and to minimize stromal scarring (Fig. 8).

In clinical cases of RCE, the epithelium often will be irregular and edematous. For this reason, the experiments in this study were performed by removing the epithelium prior to laser application. For clinical use, we recommend focally debriding the loose epithelium with a cellulose sponge. In cases where the epithelium often will be irregular and edematous. For this reason, the experiments in this study were performed by removing the epithelium prior to laser application. For clinical use, we recommend focally debriding the loose epithelium with a cellulose sponge. In cases where the epithelium often will be irregular and edematous.
epithelium is not swollen, debridement may not be necessary. The red helium–neon laser spot must be critically focused on the basement membrane or just anterior to it to avoid inadvertent stromal punctures, which have been shown to produce stromal scars.\(^\text{15}\) Although one can focus the laser under diffuse illumination, it is easier to focus the red laser spot with the cobalt blue background light. A contact lens is not necessary to focus the light on the basement membrane. At 2.0 mJ, one can hear little “snaps” as the laser shock-wave hits the cornea. It is possible that the generated shock-wave could rupture Descemet’s membrane. However, rupturing was seen in only 2% of the specimens, and any endothelial cell loss should be quite minimal. The laser spots were placed approximately 0.25 mm apart. One may anticipate that patients will need retreatments in areas that were missed on the first treatment session, as was seen with the microdiathermy technique.\(^\text{1}\) Energy levels between 1.5 and 2.5 mJ appear appropriate for human corneas.

The mechanism for improved adherence of epithelial cells after stromal puncture has not been completely determined. However, there are apparent morphologic similarities between the Nd:YAG treatment and the other puncture techniques. Wood and co-investigators showed that a new connective tissue layer formed in a rabbit cornea 4 weeks after microdiathermy, which allowed new basal lamina and basement membrane complexes to form.\(^\text{1,3}\) Disrupted collagen bundles underlying the epithelial layer were seen in the Nd:YAG treated rabbit model at 4 weeks (Fig. 5). Needle stromal puncture caused new basement membrane formation by 4 weeks without reduplication, as is seen after microdiathermy.\(^\text{16}\) In the Nd:YAG treated rabbits, the basement membrane reformation was almost complete by 4 weeks and was intact by 3 months. No areas of reduplication were found. The reestablishment of an intact basement membrane would be crucial for successful treatment of RCE. The fact that the collagen fibers below the stromal break appeared to be normal shows that the Nd:YAG laser technique can create superficial breaks without posterior involvement.

Key words: recurrent erosion, Nd:YAG laser, stromal puncture, corneal wound healing, electron microscopy

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References