Changes in Aqueous Outflow After In Vitro Neodymium: Yttrium Aluminum Garnet Laser Cyclophotocoagulation

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To examine the possible role of transscleral outflow routes, enucleated human and porcine eyes underwent noncontact neodymium: yttrium aluminum garnet (Nd:YAG) laser cyclophotocoagulation 3 mm posterior to the limbus. Pars plana lesions were verified histologically. The eyes were perfused with saline solution at 50 mm Hg perfusion pressure, placing the tip of the needle into the hyaloid orbital space. The outflow facility was 0.072 μl/min/mm Hg in paired controls and 0.105 μl/min/mm Hg in human lasered eyes, a difference of 31%. In porcine eyes the difference was 43%. Since concepts of aqueous production, impaired circulation, and inflammation do not apply to enucleated eyes, the increase may be related to pars plana transscleral flow facilitated by disruption of the neuroepithelial barrier.

Materials and Methods

Fourteen unmatched enucleated porcine eyes, 6–8 hr old, packed in ice, were obtained from a slaughterhouse. Eyes were discarded if they showed any gross abnormalities when viewed with a hand-held magnifier. Seven eyes were selected at random and assigned to the laser group. The remaining seven unaltered eyes served as controls. Laser treatments were applied using the LASAG Microruptor II Nd:YAG laser (Thun, Switzerland) at 10 J, 20 msec, and a retrofocus of 9 (approximately 3 mm). Due to the increased pigmentation and thickness of porcine sclera, 10 J were required to obtain visible lesions comparable to those produced at 5–6 J in human eyes at the pars plana. Through preliminary experiments on porcine eyes, the distance between the limbus and the mid pars plana was measured to be an average of 4 mm. Forty laser applications were given for 360°, 4-mm posterior to the surgical limbus.

Ten pairs of matched enucleated human eyes, 1–4 days old, without gross pathologic or surgical alterations, were obtained from the Lions Eye Bank of Delaware Valley. One eye from each pair was randomly assigned to the laser group, and the other eye served as a control. Laser treatments were applied using the LASAG Microruptor II Nd:YAG laser at 5–6 J, 20 msec, and a retrofocus of 9. Forty applications were given for 360°, 3-mm posterior to the limbus, as used in some clinical studies.

Immediately after laser treatment all eyes were stabilized in a surgical tray with saline moist towelettes to prevent drying and to maintain a constant perfusion temperature of 22°C. A 30-G, ½-inch infusion needle (Becton-Dickinson, Rutherford, NJ) was inserted 2 mm from the limbus, and slowly advanced intraocularly until seen with the indirect ophthalmo-
scope. The needle was then withdrawn a distance of 7 mm, so that the tip was positioned internally close to the pigment epithelium in the porcine equivalent of the canal of Hannover. Similarly, in human eyes undergoing perfusion, the tip of the needle was inserted 3-mm posterior to the limbus and withdrawn 6 mm to enter the canal of Hannover, or the superficial anterior cortical gel. Glue was used to seal the ocular penetration site of the needle completely.

A simple quantitative perfusion apparatus consisted of a reservoir of 500 ml of normal saline solution (0.9% sodium chloride; Abbott Laboratories, North Chicago, IL) connected by polyethylene intravenous tubing (Venoset 78 with cair clamp; Abbott) to the 30-G ½-inch needle placed in the eyes. The height of the saline reservoir was varied to establish the desired intraocular pressure. A U-shaped mercury manometer tube with a custom port to accommodate the infusion needle was used to verify the pressures before each perfusion. The infusion lines were fully opened for 30 min to allow for pressure equilibration of the eyes with the infusion system. After equilibration, an air bubble was introduced (time, 0) into the intravenous infusion lines of both the lasered and the control eye, close to the saline reservoirs. The initial position of the lower air-bubble meniscus was marked (distance, 0 mm). The progression of the meniscus was measured with a 1-mm division tape measure every 15 min for 2 hr. These distances were converted to inflow volumes by determining the amount of fluid held by a given length of tubing.

Seven pairs of unmatched porcine eyes were perfused at 100 mm Hg (Fig. 1), six pairs of human eyes at 100 mm Hg (Fig. 2), and four pairs of human eyes at 50 mm Hg (Fig. 3). The perfusion lines to be connected to the lasered eye and its control were randomly selected in both porcine and human eye perfusions to reduce systematic error. During perfusion, shallowing of the anterior chamber was not noted. As in previous studies, it was assumed that global inflow equaled the sum of conventional and nonconventional outflow at steady state. Outflow values were averaged for the 15-min intervals up to 2 hr, and standard deviations were calculated. The paired t-test was used to test for statistical significance at the usual level of 0.05. After perfusion the eyes were fixed in 7% formaldehyde. They were dissected in the equatorial meridian, and pars plana burns were verified in all lasered eyes. Gross photographs were taken and representative sections were submitted for routine histology as described elsewhere.

**Results**

Compared with controls, the rate of outflow was increased by 43% in porcine eyes (Fig. 1) and by 28–31% in human eyes (Figs. 2, 3). In human eyes,
the facility of outflow at 50 mm Hg was 0.072 in controls versus 0.105 \( \mu l/min/mm Hg \) in lasered eyes, and at 100 mm Hg, it was 0.061 versus 0.085 \( \mu l/min/mm Hg \), respectively. The facility of outflow was decreased for higher pressures. All differences were statistically significant at the usual confidence levels of 0.05, two sided. Postperfusion gross examination of lasered human eyes showed pars plana lesions at the 3-mm laser focus used (Fig. 4C). Histologic sections showed disruptions of the pigmented pars plana epithelium (Fig. 4D) in experimental eyes which were not found in fellow control eyes (Figs. 4A–B). The scleral changes after noncontact Nd:YAG laser were minimal on microscopic examination.

**Discussion**

Our results showed that passive outflow of intraocular fluid was increased after in vitro conventional laser cycloablation. Perfusion of enucleated eyes has been used extensively in the past and, as in our experiment, derived its internal consistency from randomization and permutation of the experimental eyes versus paired controls.\(^9\),\(^10\) Outflow was increased by 28–43% in all lasered porcine and human eyes, making laser treatment the only independent variable which could account for the difference within each pair. It is possible that the greater effectiveness in porcine eyes was related to pigmentation and higher total energy levels as suggested by more explosive absorption during laser treatment.\(^11\),\(^12\)

The rate of outflow was low; however, it was comparable to rates reported for enucleated eyes perfused from the vitreous compartment, possibly implicating a component of vitreous in the increased resistance to outflow.\(^10\),\(^13\)

In cycloablation, four pathomechanisms of pressure lowering have been proposed. First, the destruction of aqueous-producing epithelium may reduce aqueous production.\(^1\) This concept demands exact focus of the laser on the corona ciliaris and requires more laser energy than would be needed at the pars

![Fig. 4. Posterior view of the anterior segment of lasered eyes (C, D) and control eyes (A, B) after perfusion. Note pars plana epithelial lesion in (C, D) (arrow). (B) shows a corresponding pars plana area in the control fellow eye (arrow) (H & E, original magnification \( \times 25 \)).](image-url)
cesses resulting in less absorption of light, the pigmentation of transscleral light to the epithelium, and the reduced pigmentation of the apical ciliary processes.14 Some reasons for this difference may be anatomic details may interfere with the creation of a lesion of therapeutic degree at the corona ciliaris level.15 In addition, as discussed previously, there may be no apparent damage to the aqueous-producing epithelium when applying the usual clinical focus at 2–3-mm posterior to the limbus.15

The second pathogenic theory of pressure lowering in ablation is mediation by the inflammatory process which may be more important in the acute phase.3 Two phases have been distinguished for cyclocryopexy based on increased facility of outflow.4 Mediation by prostaglandins and other eicosanoids is likely to occur to some extent in cycloablation, and it is independent of anatomic ciliary atrophy or the location of the ablation.2,3 One possible group of mediators are prostaglandins that enhance uveoscleral outflow.16

A third mechanism is vascular compromise of the ciliary circulation.1,17 This may play some role even though the uvea is rich in collaterals and most of the ciliary blood supply comes from the long posterior ciliary arteries and anterior ciliary arteries, ie, from the major arteriolar circle of the ciliary body. This mechanism would apply only when the arteriolar circle is photoablated extensively, requiring a focus much more anterior than that commonly used. Anterior focus with short exposures may account for hyphema and other hemorrhagic complications.18

The fourth mechanism is pressure-dependent passive outflow through the sclera: this has been postulated for ultrasound4,6 and laser application.2,3,7 Increased outflow was actually measured after cryoablation, however, this was ascribed to alterations in the trabecular meshwork.4 It has not been determined whether histologic scleral alterations are needed to increase the transscleral rate of flow to a range where it would make a therapeutic difference. The volumetric rate of flow would depend on both pressure head and scleral changes. The fact that cycloablation is effective without apparent scleral damage suggests that scleral changes may be an adjunct in the lowering of pressure. In our in vitro study histologic scleral damage was minimal, yet increased outflow was present suggesting that the neuroepithelium and not the sclera may be the main barrier to passive outflow. The pigment epithelium (PE) is the target of transscleral photoablation at the pars plana since absorption of Nd:YAG light is pigment dependent.11,12 Peyman19,20 examined the role of the PE as a barrier in posterior segment laser applications, and Quigley21 showed permanent disruption of the tight junctional barriers after cryopexy in the primate. The retina and sclera are permeable to aqueous and allow for passive outflow.22,23

In conclusion, increased fluid outflow was found in vitro under high pressure heads in eyes with laser-induced pars plana lesions. Concepts of aqueous production, impaired circulation, and inflammation do not apply to enucleated eyes. Therefore, the increase may be related to transscleral outflow routes, facilitated by disruption of the neuroepithelial barrier at the pars plana level. Such laser-induced lesions may be a contributing factor in the long-term lowering of pressure in severely glaucomatous eyes.

Key words: aqueous outflow, cyclodestruction, noncontact Nd:YAG laser, pars plana photoablation

References


