Demonstration of Aqueous Streaming Through a Laser Iridotomy Window Against the Corneal Endothelium

Yasuaki Yamamoto, MD; Toshihiko Uno, MD, PhD; Katsumi Shisida, MD; Longquan Xue, MD; Atsushi Shiraishi, MD, PhD; Xiaodong Zheng, MD, PhD; Yuichi Ohashi, MD, PhD

Objective: To determine the pathogenesis of the bullous keratopathy that is frequently observed in patients after argon laser iridotomy (ALI) by comparing the changes in aqueous flow after ALI with those that follow peripheral iridectomy in rabbit eyes.

Methods: Silicone particles were injected into the anterior chamber of rabbit eyes as tracers to monitor aqueous flow. Particle tracking velocimetry with image analysis was used to determine the direction and speed of aqueous flow in 5 pigmented rabbits that underwent ALI and 5 that underwent peripheral iridectomy.

Results: In the ALI group, silicone particles were found to stream through the iridotomy window against the corneal endothelium immediately after the pupil was constricted by a light stimulus. The mean ± SD speed of the particles was 2.97 ± 1.51 mm/s. In contrast, the mean ± SD flow rate through the iridectomy window in the peripheral iridectomy group was significantly slower at 0.36 ± 0.30 mm/s (P = .01).

Conclusion: Constriction of the pupil elicited marked aqueous streaming through the ALI window against the corneal endothelium.

Clinical Relevance: The mechanical stress to the corneal endothelium by the abnormal aqueous stream may be partially responsible for the corneal decompensation that follows ALI.

Arch Ophthalmol. 2006;124:387-393

ARGON LASER IRIDOTOMY (ALI) has been widely used for the prevention and treatment of angle closure glaucoma since its introduction into clinical practice by Quigley1 in 1981. In 1984, Pollack2 reported the first case of irreversible corneal edema after ALI, and in 1988, Schwartz et al3 reported more cases of phakic bullous keratopathy and concluded that corneal edema was a serious complication of ALI. The incidence of this devastating disorder is increasing yearly, and, in Japan, ALI-induced bullous keratopathy is now recognized as the second most common corneal disease requiring penetrating keratoplasty to restore good visual acuity.2-11

A variety of causes have been postulated for bullous keratopathy, for example, excessive laser irradiation,3,4,7-9,11 a history of acute glaucomatous attack,3,5,7,8,10,11 diabetes mellitus,3,6,8,11 and abnormalities in the corneal endothelium, such as cornea guttata and Fuch's corneal degeneration.3,6,8,9,11 However, these factors do not fully explain the pathogenetic mechanism in most cases. We hypothesize that changes in the dynamics of the flow of aqueous humor are related to the corneal endothelial decompensation that follows ALI. To date, the dynamic changes in aqueous flow in the anterior chamber have not been studied in detail because aqueous flow is not visible except when inflammatory cells are present in the anterior chamber. Recent improvements in imaging devices and computer techniques now allow the use of principles developed for particle tracking velocimetry (PTV) to detect the flow of the aqueous humor.12,13 Using silicone powder as a tracer, we quantitatively analyzed changes in the direction and speed of aqueous flow and compared the velocity and course of aqueous flow after ALI vs after peripheral iridectomy (PI) in rabbits.

Author Affiliations:
Departments of Ophthalmology, Ehime University, Shitsukawa, Toon-city, Ehime (Drs Yamamoto, Uno, Shisida, Xue, Shiraishi, Zheng, and Ohashi), and Takanoko Hospital, Matsuyama City (Drs Yamamoto and Shiraishi), Japan.

ANIMALS AND TRACER

Healthy Dutch pigmented rabbits (weight, 1.5-2.5 kg) were used. All the procedures were performed under general anesthesia with an intramuscular injection of 5% ketamine.
hydrochloride, 25 mg/kg (Ketalar; Sankyo-Yell, Tokyo, Japan), and xylazine hydrochloride, 5 mg/kg (Celactar; Bayer, Tokyo), and under topical corneal anesthesia with 0.4% oxybuprocaine hydrochloride ophthalmic solution. In managing the rabbits, we adhered strictly to the Guiding Principles in the Care and Use of Animals (US Department of Health, Education, and Welfare publication NIH 80-23).

Silicone powder (KMP-602; Shin-Etsu Chemical Co Ltd, Tokyo), consisting of homogeneous particles with a mean diameter of 30 µm and specific gravity of 0.98, was used as a tracer to make aqueous flow visible.

**EXPERIMENTAL DESIGN**

Fifteen rabbits were randomly separated into 3 equal groups. One group served as controls without treatment, the second group underwent unilateral ALI, and the third group underwent unilateral PI. The rabbits in the ALI and PI groups were monitored for more than 2 weeks until the inflammatory reactions induced by the surgical procedures were completely resolved.

To examine the pattern and velocity of aqueous flow, 0.05 g of silicone powder was suspended in 10 mL of isotonic sodium chloride solution. Then, 0.2 mL of the suspension was injected through a corneal limbal incision at the 2-o’clock position using a 30-gauge needle on a 1-mL disposable syringe. All the rabbits were allowed to recover for at least 15 minutes after the injection, until the movements of the silicone particles became consistent. Then, the movements of the silicone particles were videotaped and analyzed using PTV.

In the control group, the speed of the thermal current was quantitatively analyzed, and in the ALI and PI groups, the movement of the tracer particles in the vicinity of the iridotomy or

![Figure 1. Still photographs of particles in the anterior chamber at 0 (A), 3 (B), 5 (C), and 8 (D) seconds showing a descending particle (a), the center of the circular current (b), and an ascending particle (c). Particles attached to the surface of the lens are used to track the movement of the rabbit (d). The wavy lines in D indicate the track of each particle.](https://jamanetwork.com/)

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iridectomy window was also measured. To induce pupillary constriction, the width of the slit beam was increased from 0.3 to 2.0 mm. Changes in aqueous flow were monitored before and during miosis. The speed of flow of the aqueous from the posterior chamber through the opening in the iris into the anterior chamber was compared in the 2 groups.

EXAMINATION OF AQUEOUS FLOW AND PTV

Movement of the silicone particles into the anterior chamber was observed by using a slitlamp biomicroscope and was recorded on videotape by using a charge-coupled device camera. The slit beam was aligned with the center of the cornea and projected vertically onto the anterior surface of the lens. The angle between the observer’s eye and the slit beam was maintained at 60 degrees.

The images of the movement of the particles on the videotape were transferred to a personal computer, and the flow speed was determined by monitoring the displacement of individual particles using image analysis software (Image Tracker PTV 2001; Digimo Co Ltd, Osaka, Japan). The images were first converted into still pictures at 30 frames per second, and the coordinates of selected particles were determined for each frame. Then, regression lines were calculated based on the sequential changes of the coordinates of each particle. The speed of flow of the particles was calculated from the slope of each regression line.

Because the coordinates of the particles also changed from movements of the rabbit, the coordinates of each particle were corrected by subtracting the coordinates of particles that were adherent to the surface of the lens or corneal endothelium from those of the individual particles floating in the aqueous humor.

SURGICAL PROCEDURES FOR ALI AND PI

The multicolor laser photocoagulator (Novus Omni; Coherent, Inc, Santa Clara, Calif) was used for ALI. The 2-step long-pulse technique using the Abraham lens was used: 8 stretching burns with 521 nm of argon green, 200-µm spot size, 0.2 second, and 200 mW of power followed by 272 to 432 penetrating burns with 521 nm of argon green, 50-µm spot size, 0.02 second, and 1000 mW of power. For PI, the iris was pulled out of a limbal incision and resected to produce an iridectomy diameter of approximately 3.0 mm at the 12-o’clock position. The prolapsed iris was gently repositioned using a spatula to avoid any incarceration in the incision site. Finally, the wound was closed using a single 10-0 nylon suture.

The silicone particles were well dispersed in the aqueous humor without any aggregation. Some particles were adherent to the corneal endothelium and the anterior surface of the lens and iris, but most were seen to be moving along with the aqueous flow.

AQUEOUS FLOW IN THE ANTERIOR CHAMBER

Control Group

Slitlamp examination showed continuous movement of the particles in a circular path, with a descending flow near the corneal endothelium and an ascending flow near the anterior surface of the iris. This pattern is consistent with the theory of the thermal current of the aqueous humor in the anterior chamber (Figure 1). Changes in the coordinates of 19 particles in 5 rabbit eyes were plotted. The graph of 1 descending particle in the vicinity of the corneal endothelium is shown in Figure 2. The mean±SD flow speeds were 0.180±0.056 mm/s for the descending flow near the corneal endothelium and 0.068±0.015 mm/s for the ascending flow near the anterior surface of the lens.

ALI Group

The pattern of thermal flow of the particles was almost the same as in control eyes. However, when the pupil was constricted, a stream of particles was seen to flow into the anterior chamber from the posterior chamber through the iridotomy window. After the pupil dilated, the particles were drawn back into the posterior chamber through the iridotomy window. The tracking images of these particles, plotted by monitoring individual particles for 1 second, are shown in Figure 3 and Figure 4. The streaming images were seen to collide against the corneal endothelium and then move downward in the anterior chamber (arrows in Figure 3).

PI Group

In the PI group, particles were seen to descend not only near the cornea but also along the anterior surface of the lens. The ascending particles were seen near the surface of the iris when the slit beam was shifted laterally. Constriction of the pupil accelerated the speed of the descending particles near the iridotomy window to some extent, but no particles were seen to collide with the corneal endothelium. The tracking images of these particles, plotted for 1 second, are shown in Figure 5.
COMPARISON OF FLOW THROUGH THE OPEN WINDOW IN THE IRIS AFTER MIOSIS IN BOTH GROUPS

The speed of the particles streaming through the opening in the ALI and PI group during a 0.1-second period after miosis is plotted in Figure 6. The mean ± SD flow speed of the 25 particles in the ALI group was 2.97 ± 1.51 mm/s, whereas that of the 25 particles in the PI group was 0.36 ± 0.30 mm/s. This difference in the mean flow speed was significant (P = .01, by Mann-Whitney test). The size of the iris window was measured on the images downloaded to the computer. The mean ± SD area of laser iridotomy in the ALI group was 0.96 ± 0.31 mm², whereas that in the PI group was 5.84 ± 2.12 mm². The difference in the areas of the iris window between the 2 groups was significant (P = .006, by Mann-Whitney test).

COMMENT

We succeeded in “seeing” the flow of aqueous humor in the anterior chamber of rabbit eyes by using silicone powder as a tracer. The technique of PTV was then used to determine the direction and speed of the particles. Our results confirm the presence of a thermal current of aqueous humor in the anterior chamber. As stated, the accuracy of the velocity measurement by PTV is ultimately determined by the ability of the scattering particles to follow the instantaneous motion of the fluid.

In this sense, careful selection of a tracer particle is critical, and several properties should be present in the particles. First, the tracer particles must be large enough to have good light-scattering ability and to be detected by a video camera so that digitized data can be analyzed. Second, the tracer particle should be small enough to be carried passively with the current of the fluid. An ideal tracer particle would have a specific gravity identical to that of the aqueous humor. And third, the particle should be nontoxic, noncorrosive, and chemically inert. With respect to these requirements, many studies have used particles with diameters ranging from 2 to 500 µm and density ratios of 0.7 to 1.05.15 Silicone powder has these properties and thus was used in this study.

The exact cause of the corneal endothelial decompensation that follows ALI has not been determined. For example, excessive laser irradiation can cause serious endothelial damage, but we seldom see such a
patient develop severe corneal edema immediately after surgery. In addition, corneal endothelial decompensation can develop even after uneventful laser procedures. Although a history of acute glaucomatous attack is a risk factor for corneal endothelial decompensation, irreversible bullous keratopathy almost never develops in eyes that undergo surgical iridectomy. The presence of diabetes mellitus or corneal endothelial abnormalities, such as cornea guttata or Fuchs corneal dystrophy, has not been associated with many cases of bullous keratopathy.

Our most striking finding was the presence of aqueous streaming through the small opening of the iris after ALI. This showed that laser iridotomy causes an extremely fast forward aqueous flow, which occurs through the iris window during miosis, followed by the backward flow as the pupil dilates. The mean speed of this streaming from the posterior chamber to the anterior chamber was estimated to be 2.97 mm/s, which is approximately 17 times faster than the ordinary thermal current. More important, the stream was directed against the corneal endothelium, unlike the direction of the physiologic thermal current, which flows parallel to the corneal endothelium. Just as the continuous shear stress of vascular flow on vascular endothelial cells has gained attention as a causative mechanism in arteriosclerosis or aneurysm formation,16-18 such an abnormal stream can be suggested to damage corneal endothelial cells. If this streaming is repeated in response to light stimuli for long periods, the corneal endothelial cells around the window may be damaged, leading to corneal endothelial cell dysfunction.

In contrast to the stream observed in the ALI group, the flow speed through a large-diameter surgical iridectomy in the PI group was found to be slow and not directed against the corneal endothelium. This may explain the fewer cases of endothelial dysfunction observed in patients after standard surgical PI, a procedure widely performed before the development of ALI.

Corneal endothelial dysfunction occurs predominantly after ALI but also rarely after Nd:YAG laser iridotomy. One may argue that such corneal endothelial decompensation can be avoided if an Nd:YAG laser is applied to make an iris opening because the thermal effect is thought to be substantially reduced using this procedure. However, it has been reported that the dysfunction can develop in patients after laser iridotomy using an Nd:YAG laser.7,9,19,20 Therefore, it seems that the thermal damage to the corneal endothelium elicited by the ALI procedure cannot be the only causative factor, and...
Figure 5. Photograph demonstrating the aqueous flow through the peripheral iridectomy (PI) window. A, An eye with PI during pupil constriction (when the slit beam was increased). B, At that time, the tracking images of particles are indicated by the higher view in the vicinity of the iridectomy window (arrowheads). None of the particles were noted to collide with the corneal endothelium. The wavy lines indicate the track of each particle.

Figure 6. The speed of the particles streaming through the opening in the argon laser iridotomy (ALI) and peripheral iridectomy (PI) groups during a 0.1-second period after miosis. The mean±SD flow speed in the ALI group was 2.97±1.51 mm/s and in the PI group was 0.36±0.30 mm/s (P=.01, by Mann-Whitney test).
Why does such streaming occur? Generally, during miosis, the pressure in the posterior chamber rises, which leads to the flow of aqueous into the anterior chamber. As the pupil and the central region of the posterior surface of the lens make appositional contact with the anterior surface of the lens and retard aqueous flow, the rapid release of the pressure should result in the aqueous stream when the opening is small.

Because the aqueous humor plays an essential role in maintaining the homeostasis of the anterior segment of the eye, any changes may have a profound effect and could be associated with a variety of ocular disorders. In this regard, the method of making aqueous flow visible described herein can be used to analyze changes in its dynamics and could possibly contribute to the further understanding of the pathogenesis of anterior segment disorders.

Submitted for Publication: April 20, 2005; accepted July 15, 2005.

Correspondence: Yasuaki Yamamoto, MD, Department of Ophthalmology, Ehime University, Shitsukawa, Toon-city, Ehime, Japan (yasuaki@m.ehime-u.ac.jp).

Financial Disclosure: None.