Investigation of Corneal Ablation Efficiency Using Ultraviolet 213-nm Solid State Laser Pulses

Geoffrey T. Dair, Wayne S. Pelouch, Paul P. van Saarloos, Darren J. Lloyd, Simone M. Paz Linares, and Fred Reinholz

Purpose. To determine the threshold and efficiency of corneal ablation for various values of laser fluence at the ultraviolet wavelength of 213 nm.

Methods. A commercial Q-switched Nd:YAG laser was used to produce the fifth harmonic wavelength of 213 nm. Ablation trials were carried out on porcine corneas. Slit ablations of dimensions 0.5 × 2.5 mm were performed using seven values of laser fluence to obtain the most efficient fluence for ablation. The morphology of each ablation was obtained using a computer-automated confocal profiling system. These profiles were then analyzed to determine the ablation depth for the range of fluence values used.

Results. A fluence in the region of 200 mJ/cm^2 was found to be the most efficient for ablation. The efficiency in this region was approximately 0.35 mm^3/J, and the ablation rate was found to be 0.6 μm/pulse. The ablation threshold was found to occur at a fluence of 50 mJ/cm^2. In the region of highest efficiency, the peak varied slightly in the fluence range between 150 and 250 mJ/cm^2.

Conclusions. This study confirms that the corneal ablation properties at 213 nm are comparable with those at the 193-nm excimer laser wavelength. Increased pulse energy was obtained for the fifth harmonic of Nd:YAG lasers at 213 nm through the use of new nonlinear optical crystals to perform the frequency conversion. A solid state laser is feasible to replace the excimer gas laser for performing refractive surgery procedures. For the first time, the increased energy at 213 nm allows large-beam ablations to be performed at this wavelength. (*Invest Ophthalmol Vis Sci.* 1999;40:2752–2756)

With the success of the excimer laser wavelength of 193 nm to ablate corneal tissue,[1,2] it became desirable to develop a solid state laser that could operate in the UV region. This desire was born as a result of the problems associated with gas lasers, including the use of the toxic gas fluorine,[3] which is a safety issue in the clinical environment. In contrast, solid state can provide increased reliability, robustness of design, safety, and lower operating costs than gas or dye lasers. Although solid state lasing mediums generally operate in the visible to infrared region, nonlinear optical crystals have been used to frequency convert these laser outputs to UV wavelengths.

The Nd:YAG laser has emerged as a possible candidate to replace the excimer gas laser. The fundamental wavelength of this laser is 1064 nm, and three nonlinear optical crystals can be used to produce the fifth harmonic wavelength of 213 nm. As a result, a number of different configurations have been proposed using various combinations of nonlinear crystals. Configurations incorporating the two crystals deuterated cesium dihydrogen arsenate (CD*A) and β-barium borate (BBO)[4] are subject to very low percentage conversion efficiencies.[5,6] This has limited the available energy at 213 nm, and, consequently, the laser beam has had to be focused on a small spot to achieve the fluences required for ablation. With the invention of the nonlinear crystal cesium lithium borate (CLBO)[7] the overall conversion efficiency of this process has been greatly improved, and the amount of available energy at 213 nm has been increased.[8] This increase in energy has enabled the development of a solid state UV laser with the potential to perform ablations using a large beam diameter.

Previous studies using solid state lasers investigating the similarity in ablation properties between the 193-nm excimer wavelength and the 213-nm wavelength of solid state lasers have been constrained to use of low-output pulse energies.[5,6,9–11] These studies were restricted to using small beam diameters of 0.5 mm or less to achieve a fluence above the ablation threshold.

We present a quantitative analysis of the ablation rate using a solid state laser with increased pulse energy. From the ablation rate data, ablation efficiency is calculated, and a determination of the most efficient fluence for tissue removal is made.

Materials and Methods

The laser engine used to perform the ablations was a Q-switched, flashlamp pumped Nd:YAG laser (Surelite II; Continuum, Santa Clara, CA) capable of producing up to 660 mJ of energy per pulse at a wavelength of 1064 nm. The duration of the 1064-nm pulses was 5 nsec with a repetition rate of 10 Hz. To produce the fifth harmonic wavelength, three nonlinear crystals were used. The second harmonic (532 nm) was produced using the crystal BBO (Casix Inc., Fuzhou, China). The fourth (266 nm) and fifth harmonics (213 nm) were produced using CLBO crystals (Crystal Associates, Waldwick, New Jersey). The conversion efficiencies obtained were 60%, 25%, and 40% respectively, for each stage, resulting in an overall conversion efficiency of 6%. The maximum fifth harmonic output energy was 20 mJ per pulse for a fundamental input energy of 330 mJ.

Ablations were carried out on porcine corneas obtained from an abattoir. All eyes were used within 5 hours of enucleation and stored on ice until required for ablation. Immediately before laser irradiation the epithelium was débrided and the eye vacuum stabilized onto a clamped syringe. The eye was positioned less than 5 mm behind a 0.5 × 2.5-mm slit onto which the collimated laser beam was centered. Therefore, the slit acted as an aperture for the beam rather than using focusing to produce the required beam diameter. The dimensions of the

From the Lions Eye Institute, Center for Ophthalmology and Visual Science, University of Western Australia, Nedlands.

Submitted for publication February 5, 1999; accepted April 29, 1999.

Commercial relationships policy: P.

Corresponding author: Geoffrey T. Dair, Lions Eye Institute, 2 Verdun Street, Nedlands 6009, Perth, Western Australia.
E-mail: gdair@cyllene.uwa.edu.au

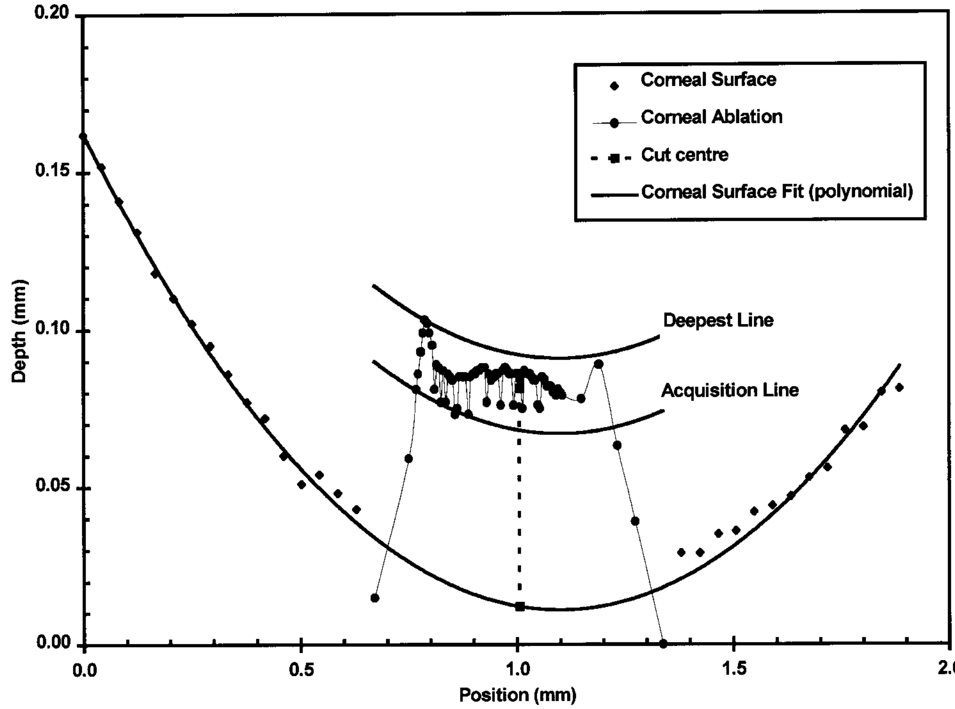

FIGURE 1. Typical confocal ablation profile. Profile represents ablation with 100 pulses at a fluence of 210 mJ/cm^2. Ablation depth for this profile was 69 μm yielding an ablation rate of 0.69 μm/pulse.

slit were chosen to produce a rectangular ablation profile to simplify the task of measuring the depth. The pulse energy through the 1.25-mm^2 slit was measured before and after each ablation using a power meter (model 1825-C; Newport, Santa Ana, CA) with a detector (model 818T-10; Newport). A small fan was used for each ablation to disperse the ablation plume to minimize any absorbing or blocking effect this would have on the impinging laser radiation.[12,13]

For each ablation trial, the fluence was held constant, and a number of eyes were ablated with a varying number of pulses. This allowed the ablation rate, defined as the etch depth per pulse to be obtained for a wide range of fluence values. This procedure was repeated for fluence values of 80, 120, 150, 180, 210, and 250 mJ/cm^2. The number of pulses was varied between 100 and 400. A separate trial was undertaken to determine the fluence value for the threshold of ablation. The method used for this trial was to irradiate eyes with low values of fluence and a large number of pulses until a notable etching effect was observed from a profiling system (described later).

In an effort to eliminate potential depth measurement errors associated with histologic methods,[14,15] the ablated eyes were analyzed immediately after ablation. Surface profiles of the ablated corneas across the 0.5-mm cut were measured using a laboratory custom-made, computer-automated confocal profiling system. The design of the profiling system is similar to a confocal microscope and has a depth resolution of 10 μm. Although the resolution is greater than the submicrometer ablation of each pulse, it is suitable for measuring the depth of an ablation resulting from a large number of pulses. Also, a resolution of this magnitude provided a convenient working distance and facilitated surface detection. Calibration of the profiler was achieved using an interferometric method. This involved using the actuator that was used to oscillate the microscope objective to oscillate a plane mirror in one arm of an interferometer. This allowed accurate calibration of the actuator position. Immediately after irradiation, the eye was placed in the profiler while affixed to the syringe to maintain intraocular pressure. The eye and syringe were placed on an x–y translation stage (Newport) and positioned in the beam of the profiler so that it was at the latitudinal center of the cut. The microscope objective used to focus the beam onto the surface of the cornea was oscillated with a frequency of 25 Hz. This enabled the depth measurement at each position across the cut to be averaged over many measurements to minimize error. The profile data were then analyzed to obtain the depth of ablation.

The corneal profile data obtained from the confocal microscope were separated into two data series. The first represented the unablated corneal profile. The second series represented the ablated segment of the cornea. Additional data series were then derived and used in the calculation of ablation depth.

Figure 1 shows one such profile. To obtain a meaningful measurement of the ablation depth, the need arose to extrapolate the unablated curvature of the corneal surface. In this way, the depth could be calculated from the surface extrapolation rather than from a straight line across each edge of the cut. This extrapolated curve was determined by applying a second-order polynomial fit to the corneal surface data points. To calculate the depth of ablation, another two polynomials were used. Each of these polynomials was parallel to the fitted curve of the unablated corneal surface. The first was drawn through the deepest point of the ablated region. The second is termed an acquisition line and was heuristically set at 75% of the distance between the surface fit and the deepest point of the ablation. The acquisition line determines which data points from the ablated region are included or acquired for calculation of the cut depth. Included points are therefore those located between the deepest point and the acquisition lines. The magnitude of the depth of each included point was taken as the vertical distance from the corneal surface fit. Those data

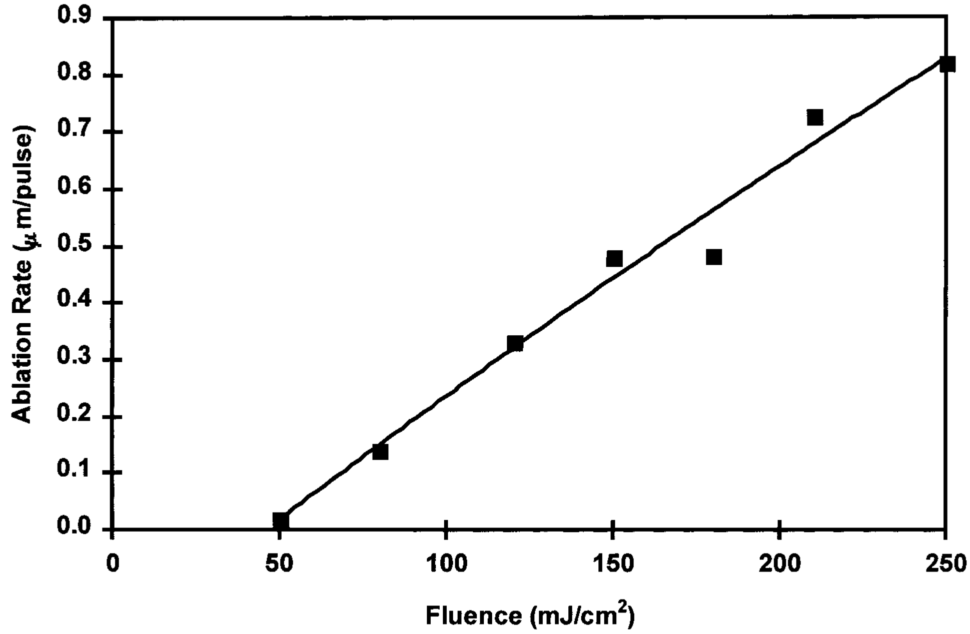

FIGURE 2. Plot of ablation rate versus fluence, displaying an almost linear relationship between etch depth per pulse and fluence used.

points that are included are then averaged and an ablation depth obtained. A line displaying the averaged ablation depth is located in the central region of the cut in Figure 1.

RESULTS

For the fluence values for which the number of pulses was varied, the etch depth was observed to increase with increasing pulse numbers yielding a linear relationship between etch depth and number of pulses for constant fluence. For each fluence value, the slope of the linear plot of etch depth versus pulse number was used to obtain the ablation rate.

Figure 2 shows the relationship of ablation rate versus fluence. For the range of fluence values used in the study, this relationship appears to be almost linear. The threshold of ablation was found to occur for a fluence of 50 mJ/cm^2. This fluence produced a cut depth of 25 μm after exposure to 1200 pulses corresponding to an ablation rate of 0.02 μm/pulse. The observed cut depth at this fluence is approximately double the resolution of the confocal profiling system. In the fluence range between 150 and 250 mJ/cm^2 the rate increases to 0.45 and 0.8 μm/pulse. From these results, the ablation efficiency was calculated for each fluence.

Ablation efficiency for a given fluence can be obtained by dividing the ablation rate by the value of fluence. The plot of ablation efficiency with fluence is shown in Figure 3. The plot shows that a plateau in efficiency was reached at a fluence of 150 mJ/cm^2. This occurred for an efficiency value of 0.32

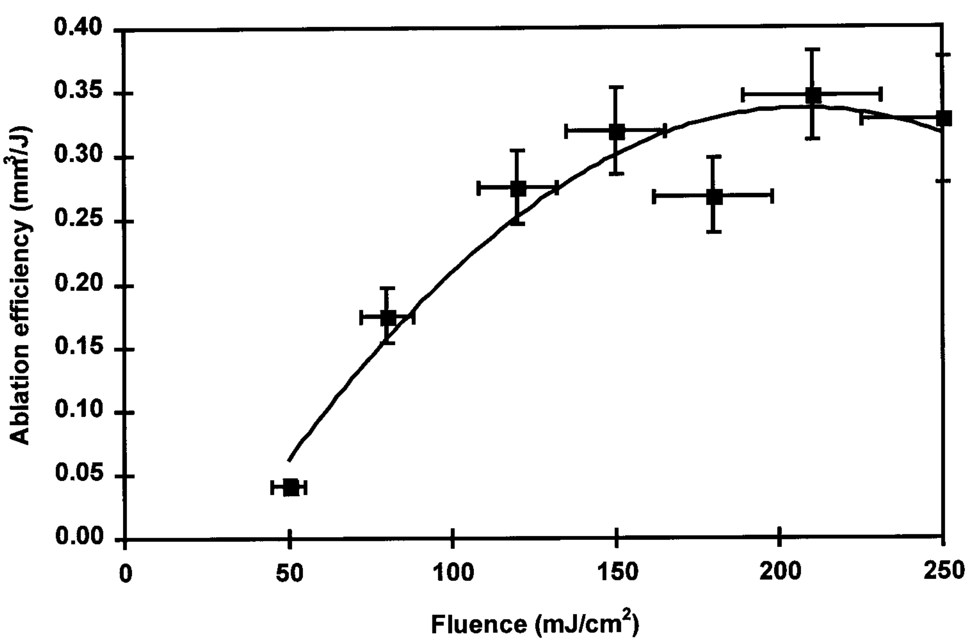

FIGURE 3. Plot of ablation efficiency versus fluence. The plot shows two regions of interest on either side of the fluence value 150 mJ/cm^2: a steeply increasing function from the ablation threshold fluence and a plateau region for higher fluence values.

mm^3/J. For fluences above this value up to the maximum used of 250 mJ/cm^2 only a small fluctuation in efficiency was observed between 0.32 and 0.35 mm^3/J. In the range from ablation threshold fluence (50 mJ/cm^2) up to the fluence where the efficiency reaches a plateau, the efficiency increases steeply, from less than 0.05 mm^3/J to 0.32 mm^3/J.

An error analysis was performed on each of the calculated points for ablation efficiency, and results are shown in Figure 3 as error bars. The error in ablation efficiency was obtained by combining the error in ablation rate with the uncertainty of the fluence. The error in the ablation rate was found from the SE in the linear regression gradient of the ablation depth versus pulse number plot obtained for each fluence. Uncertainty in the measurement of fluence emerged as the dominant quantity in the determination of the combined error. It can be seen that the curve fit of ablation efficiency lies within the estimated error of data points.

Discussion

The absorption of the cornea in the wavelength range from 190 nm to approximately 220 nm remains relatively constant.[16] This suggests that solid state laser wavelengths in this range could be suitable for performing corneal ablation and could achieve ablation rates comparable with those of the excimer laser. Previous ablation studies[2,15] using the excimer wavelength of 193 nm achieved rates of 0.45μm/pulse at a fluence of 200 mJ/cm^2.

An early study by Ren et al.[5] using a solid state laser at 213 nm used a larger fluence (1.2 J/cm^2) than was used in this study. The ablation rate was obtained by counting the number of pulses required to perforate the cornea. The laser energy used was 0.4 mJ/pulse in a spot size of 0.2-mm diameter. The ablation rate at this fluence was estimated to be 0.3 μm/pulse. This is a lower rate than obtained in this study, even though the fluence value was more than four times the largest fluence we used.

In more recent study Caughey et al.[4] estimated the ablation rate in porcine corneas to be 0.4 μm/pulse at a fluence of 200 mJ/cm2. This work was also performed at the 213 nm wavelength with a maximum pulse energy of 1.1 mJ and beam diameters between 0.5 and 1.1 mm. Depth analysis was performed by focusing a microscope on the top and bottom sections of the cut. Our results at this fluence show an agreement with this value to within 50%.

These studies showed that the 213-nm wavelength produces tissue ablation without damage to the surrounding tissue in a fashion similar to the results achieved with the 193-nm excimer laser. The observations in Caughey et al. of surrounding tissue damage were made using histologic methods.

Further work by Shen et al.[17] on the ablation rate of human cornea at 213 nm showed a relationship between ablation rate and fluence similar to our results. However, the ablation rates at comparable fluence values used in our study were consistently lower. They also used perforation of a section of tissue of known thickness to obtain the ablation rate.

The first to investigate the most efficient fluence for operation at the 193-nm wavelength were Krueger and Trokel.[2] Ablation rates were found by measuring the number of pulses required to achieve corneal perforation for a wide range of fluences. From these data the fluence for highest efficiency was found by calculating the amount of radiant energy that was required to ablate 100 μm of tissue. A refinement to this method was made in later work that quantified ablation efficiency as the volume of tissue removed per unit of input energy.[15] The importance of calculating ablation efficiency can be seen in terms of the amount of energy required to remove a given volume of tissue. A less efficient fluence requires more energy than is necessary to remove the same volume of tissue. As a result, a greater proportion of the energy must be spent on processes other than tissue removal. These processes may be manifest in increased heating of the cornea or a larger shock wave on the surface of the cornea.

We were able to characterize the ablation behavior of the solid state laser (Fig. 1). The variation of the data in the ablated area suggests that the beam used in this study was not uniform. It should be noted, however, that no beam-smoothing techniques in the form of image rotation or spatial filtering were used. We then calculated the ablation efficiency in a range similar to that used in current clinical excimer lasers. The efficiency was obtained by dividing the ablation rate by the fluence and had units of joules per cubic millimeter. The results shown in Figure 3 suggest that the efficiency reaches a maximum for fluence values above 150 mJ/cm^2. The work of Krueger et al.[2] was performed on bovine corneas, and they found the most efficient fluence for ablation at 193 nm was 200 mJ/cm^2. Others have found the most efficient fluence to be within the range of 150 to 400 mJ/cm^2.[15] The ablation rate at these fluences was approximately 0.4 μm/pulse, which is slightly lower than the value we obtained of 0.48 μm/pulse (at 150 mJ/cm^2) although within 20%. The fluence value of 180 mJ/cm^2 used with clinical excimer lasers falls within this range.

In addition to the laser parameters used in this study, there is scope to investigate the effect of varying other parameters and the effects this would have on ablation at this new wavelength. These include the effects of pulse duration and repetition rate. Pulse duration has been shown to affect ablation threshold. Using a pulse duration of 25 psec, Hu and Juhasz[18] observed an ablation threshold fluence of 3 mJ/cm^2 in human corneas. This was achieved using a wavelength of 211 nm and a repetition rate of 1 kHz. This repetition rate represents an increase of two orders of magnitude above the 10-Hz rate used by excimer lasers, and it is therefore difficult to quantify the effect. A limited study of the effect of repetition rate on ablation threshold was conducted for rates of 1, 10, and 25 Hz by Krueger et al.[1] using 193 nm. The results showed there was no change in ablation threshold over this range. This is yet to be established at even higher rates in the kilohertz regimen and the longer 213-nm wavelength.

This study confirms that corneal ablation rates at 213 nm are comparable with those achieved with excimer lasers at 193 nm for similar pulse duration and repetition rates. A solid state laser with increased energy at 213 nm capable of performing large-beam ablations may provide an improvement in corneal hydration[14,19] through a reduction in the time required to remove a given amount of tissue.

References

1. Krueger RR, Trokel SL, Schubert HD. Interaction of ultraviolet laser light with the cornea. *Invest Ophthalmol Vis Sci.* 1985;26:1455–1464.

2. Krueger RR, Trokel SL. Quantitation of corneal ablation by ultraviolet laser light. *Arch Ophthalmol.* 1985;103:1741–1742.
3. Sliney DH. Safety of ophthalmic excimer lasers with emphasis on compressed gases. *J Refract Corneal Surg.* 1991;7:308–314.
4. Caughey TA, Cheng F-C, Trokel SL, et al. An investigation of laser-tissue interaction of a 213 nm laser beam with animal corneas. *Lasers Light Ophthalmol.* 1994;6:77–85.
5. Ren Q, Gailitis P, Thompson KP, Lin JT. Ablation of the cornea and synthetic polymers using a uv (213 nm) solid-state laser. *IEEE J Quantum Electron.* 1990;26:2284–2288.
6. Gailitis P, Ren Q, Thompson KP, Lin JT, Waring GO. Solid state ultraviolet laser (213 nm) ablation of the cornea and synthetic collagen lenticules. *Lasers Surg Med.* 1991:556–562.
7. Mori Y, Kuroda I, Nakajima S, Sasaki T, Nakai S. New nonlinear optical crystal: cesium lithium borate. *Appl Phys Lett.* 1995;67:1818–1820.
8. Yap YK, Inagaki M, Nakajima S, Mori Y, Sasaki T. High-power fourth- and fifth-harmonic generation of a Nd:YAG laser by means of a $CsLiB_6O_{10}$. *Opt Lett.* 1996;21:1348–1350.
9. Ren Q, Simon G, Parel J. Ultraviolet solid-state laser (213-nm) photorefractive keratectomy. *Ophthalmology.* 1993;100:1828–1834.
10. Ren Q, Simon G, Parel J–M. Ultraviolet solid-state laser (213 nm) photorefractive keratectomy: in vitro study. *Ophthalmology.* 1993;100:1828–1834.
11. Ren Q, Simon G, Legeais J–M, et al. Ultraviolet solid-state laser (213 nm) photorefractive keratectomy: in vivo study. *Ophthalmology.* 1994;101:883–889.
12. Srinivasan R, Sutcliffe E. Dynamics of the ultraviolet laser ablation of corneal tissue. *Am J Ophthalmol.* 1987;103:470–471.
13. Lubatschowski H, Kermani O, Welling H, Ertmer W. A scanning and rotating slit ArF excimer laser delivery system for refractive surgery. *J Refract Surg.* 1998;14:S186–S191.
14. Dougherty PJ, Wellish KL, Maloney RK. Excimer laser ablation rate and corneal hydration. *Am J Ophthalmol.* 1994;118:169–176.
15. Van Saarloos PP, Constable IJ. Bovine corneal stroma ablation rate with 193-nm excimer laser radiation: quantitative measurement *J Refract Corneal Surg.*1990;6:424–429.
16. Lembares A, Hu X-H, Kalmus GW. Absorption spectra of corneas in the far ultraviolet region. *Invest Ophthalmol Vis Sci.* 1997;38:1283–1287.
17. Shen J-H, Joos KM, Manns F, et al. Ablation rate of PMMA and human cornea with a frequency-quintupled Nd:YAG laser (213 nm). *Lasers Surg Med.* 1997;21:179–185.
18. Hu X-H, Juhasz T. Study of corneal ablation with picosecond laser pulses at 211 nm and 263 nm. *Lasers Surg Med.* 1996;18:373–380.
19. Fields CR, Taylor SM, Barker FM. Effect of corneal edema upon the smoothness of excimer laser ablation. *Optom Vision Sci.* 1994;71:109–114.

Optic Nerve Oxygen Tension in Pigs and the Effect of Carbonic Anhydrase Inhibitors

Einar Stefánsson,[1] Peter Koch Jensen,[2] Thor Eysteinsson,[1] Kurt Bang,[3] Jens F. Kiilgaard,[2] Jens Dollerup,[3] Erik Scherfig,[2] and Morten la Cour[2]

Purpose. To evaluate how the oxygen tension of the optic nerve ($ONPo_2$) is affected by the administration of the carbonic anhydrase inhibitors dorzolamide and acetazolamide and by alterations in oxygen and carbon dioxide in the breathing mixture.

Methods. Polarographic oxygen electrodes were placed in the vitreous humor immediately over the optic disc in 20 anesthetized pigs. Blood gasses and cardiovascular physiology were monitored. $ONPo_2$ was recorded continuously with breathing gasses of 21% O_2-79% N_2, 100% O_2, 20% O_2-80% N_2, and 5.19% CO_2-19.9% O_2-74.9% N_2. Acetazolamide (15–1000 mg) and dorzolamide (6–1000 mg) were administered intravenously.

Results. The mean (± SD) $ONPo_2$ was found to be 24.1 ± 11.6 mm Hg when the pigs were breathing room air and 50.7 ± 29.3 mm Hg when they were breathing 100% O_2 ($n = 15$; $P < 0.001$). In response to breathing 5.19% CO_2, $ONPo_2$ changed from 20.8 ± 5.6 mm Hg (with 20.0% O_2) to 28.9 ± 3.6 mm Hg ($n = 4$; $P < 0.001$). Intravenous injections of 500 mg dorzolamide increased $ONPo_2$ from 16.4 ± 6.1 mm Hg to 26.9 ± 12.2 mm Hg, or 52.5% ± 21.2% ($n = 5$; $P = 0.017$). A dose-dependent effect on $ONPo_2$ was seen with intravenous dorzolamide doses of 1000, 500, 250, 125, 63, 27, 15, and 6 mg. Intravenous injections of 500 mg acetazolamide increased $ONPo_2$ from 23.6 ± 9.5 mm Hg to 30.9 ± 10.0 mm Hg ($n = 6$; $P < 0.001$), and a dose-dependent effect was seen with doses of 1000, 500, 250, 125, 31, and 15 mg.

Conclusions. $ONPo_2$ is significantly increased by the carbonic anhydrase inhibition of dorzolamide and acetazolamide, and the effect is dose dependent. These data demonstrate for the first time a direct effect of carbonic anhydrase inhibitors on $ONPo_2$. (*Invest Ophthalmol Vis Sci.* 1999;40:2756–2761)

From the [1]Department of Ophthalmology, University of Iceland; the [2]Eye Department, National University Hospital of Copenhagen, and [3]MSD Glostrup, Denmark.

Supported by The Icelandic Research Council, the University of Iceland Research Fund, The Helga Jónsdóttir and Sigurlidi Kristjánsson Memorial Fund, and MSD, Inc.

Submitted for publication December 14, 1998; revised June 8, 1999; accepted June 28, 1999.

Commercial relationships policy: E (KB, JD); C (ES, PKJ, TE, JFK, ES, MlC).

Corresponding author: Einar Stefánsson, Department of Ophthalmology, University of Iceland, Reykjavik, Iceland.
E-mail: einarste@rsp.is

Studies of optic nerve oxygen tension ($ONPo_2$) go back to Ernest in 1973,[1] and several investigators have reported on $ONPo_2$ in a number of species.[2–4] Novack et al.[5] studied the oxidative metabolism of cytochromes in the optic nerve in the cat and found it to be sensitive to arterial blood pressure, intraocular pressure, and oxygen. Cranstoun et al.[6] reported intra- and extravascular oxygen tension measurements in the pig optic nerve.

In the brain, systemically administered acetazolamide leads to increased cerebral blood flow.[7] Rassam et al.[8] found