Trabecular Meshwork Alteration and Intraocular Pressure Change Following Pulsed Near-Infrared Laser Trabeculoplasty in Cats

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BACKGROUND AND OBJECTIVE: To comparatively assess the safety and variation in intraocular pressure (IOP) of two pulsed near-infrared lasers (titanium:sapphire and alexandrite) for laser trabeculoplasty versus conventional blue-green argon laser trabeculoplasty in an animal model.

MATERIALS AND METHODS: The left eyes of 15 healthy cats received a 180° laser trabeculoplasty treatment: 5 with a titanium:sapphire laser, 5 with an alexandrite laser, and 5 with an argon laser. Preoperatively and postoperatively, all animals underwent tonometry, gonioscopy, and slit-lamp examination. The cats were observed up to 12 weeks. Scanning electron microscopy and histologic examination were performed to evaluate potential alterations in the trabecular meshwork structure.

RESULTS: IOP at 1 hour, 1 day, and 1 week following treatment was remarkably lower, irrespective of the laser source used. Following treatment with both near-infrared lasers, gonioscopy showed depigmentation underneath the area of the treated trabecular meshwork and histologic evaluation showed a decrease in pigment density. On scanning electron microscopy, damage to the trabecular meshwork structure could not be detected after treatment with near-infrared lasers.

CONCLUSIONS: Near-infrared laser trabeculoplasty was found to be effective to temporarily lower IOP in cats. The lasers selectively altered pigment-containing cells, avoiding structural damage of the trabecular meshwork anatomy.


INTRODUCTION

Laser trabeculoplasty is one of the treatment modalities for patients with glaucoma and is usually performed when topical therapy alone is insufficient. The conventional treatment is currently performed using a continuous-wave argon ion laser emitting blue-green light (488 + 514 nm wavelength). Laser trabeculoplasty using an argon laser for reduction of intraocular pressure (IOP) was first described by Wise and Witter more than 20 years ago and has since become standard for the care of patients with glaucoma.

The procedure consists of producing minute photoagulations in the trabecular meshwork. Its IOP-
lowering effect was found to decrease with time, with the patients requiring further intervention. Focal tissue damage caused by the initial heat shrinkage of the collagen and subsequent membrane formation in the chamber angle have been described to evolve from argon laser treatment. Consequently, both long-term success and the outcome of repeated procedures are unfavorable.

Recently, a new modality of laser trabeculoplasty has emerged, using a Q-switched, frequency-doubled neodymium:YAG (Nd:YAG) laser. The laser energy is selectively absorbed by melanin granules of the trabecular meshwork cells, leading to their disruption. Accordingly, the procedure was termed selective laser trabeculoplasty. Due to the very short pulse duration (3 ns), the damage by heat diffusion to the adjacent collagen tissue of the trabecular beams seems to be limited. However, because this laser emits at a relatively short wavelength of 532 nm, the treatment might be limited to superficial layers of the trabecular meshwork.

A laser operating at a longer wavelength would theoretically have the advantage of a deeper penetration depth. Therefore, both titanium:sapphire (Ti:Saphh) and alexandrite lasers, which emit in the near-infrared range (790 nm), have more recently been considered for laser trabeculoplasty. A pilot study utilizing a Ti:Saphh laser for laser trabeculoplasty in human patients with glaucoma showed promising results, demonstrating effective IOP reduction at 1-year follow-up. The purpose of this animal study was to determine variation in IOP, tissue response, and potential damage induced by two pulsed infrared lasers (Ti:Saphh and alexandrite) on the trabecular meshwork in comparison to a conventional argon laser.

MATERIALS AND METHODS

Animals

Sixteen eyes from 16 female, healthy domestic cats (Felix domesticus) with body weights between 2.9 and 3.3 kg and ages between 11 and 12 months were used for the experiments. All animals were treated in accordance with the institutional guidelines regarding animal experimentation and with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgery and examinations were performed under general anesthesia with intramuscular injection of 20 mg/kg of ketamine hydrochloride (Ketaset III; Fort Dodge Animal Health, Fort Dodge, IA), 1 mg/kg of acepromazine maleate (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO), and 0.20 mg/kg of atropine sulfate (Phoenix Scientific, Inc., St. Joseph, MO). Fifteen of the animals were randomly divided into three groups receiving unilateral trabeculoplasty treatment. Group A was treated with a Ti:Saphh laser, group B with an alexandrite laser, and group C with a conventional blue-green argon laser. One cat received a sham trabeculoplasty.

Preoperative Examination

IOP was measured on both eyes preoperatively while the cats were under general anesthesia. Additionally, proparacaine hydrochloride 0.5% was applied in both eyes for topical anesthesia. Measurements were performed using a pneumotonometer (Mentor 080, Inc., Norwell, MA). Four readings were taken on each eye. The tonometer was calibrated following the manufacturer's guidelines and a correction formula determined ex vivo by anterior chamber cannulation and a piezoelectric pressure sensor in 5 freshly enucleated cat eyes of the same species, age, and gender. Subsequently, all animals received an extensive bilateral ocular examination including biomicroscopy and gonioscopy.

Lasers

A prototype free-running Ti:Saphh (Ti:Al2O3) laser pumped by flash lamp (SOLX Inc., Boston, MA) with a wavelength of 790 nm and a pulse duration of 200 ns was used. The duration of the pulse was taken to be equal to the duration of the first relaxation oscillation. The laser beam was delivered through a 200-μm diameter fiber, which was connected to the slit lamp. The output of the fiber was refocused using a system of lenses to produce a spot with a diameter of 150 μm when focused through the gonioscopic lens and cornea. A red laser beam coupled into the fiber was used for aiming.

The prototype alexandrite (BeAl2O4·Cr3+) laser (SOLX Inc.) emitted at a wavelength of 790 nm but displayed a longer pulse duration of 1 μs. The diameter of the spot produced by that laser measured 200 μm.

For argon laser trabeculoplasty, a clinical blue-green argon laser system delivered by slit lamp (900
Table 1: Laser Parameters and Settings

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Argon</th>
<th>Ti:Sapphire</th>
<th>Alexandrite</th>
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<tbody>
<tr>
<td>Wavelength (nm)</td>
<td>488 ± 514</td>
<td>790</td>
<td>790</td>
</tr>
<tr>
<td>Pulse duration (s)</td>
<td>0.1</td>
<td>200 ns</td>
<td>1 µs</td>
</tr>
<tr>
<td>Laser spot diameter (µm)</td>
<td>50</td>
<td>150</td>
<td>200</td>
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Photocoagulator; Coherent Inc., Palo Alto, CA) emitting at 488 and 514 nm with a pulse duration of 0.1 second was used. The spot diameter at the focus was 50 µm. Laser settings and parameters are summarized in Table 1.

Laser Trabeculectomy

A special X-Y-Z carriage and a cat holder were constructed to properly position the eye of the anesthetized cat in front of the laser slit lamp. A beam-splitter was added to the slit-lamp delivery system to allow for video recording and digital photography of both treatment procedure and follow-up examinations. The left eye of each animal was treated.

A pediatric gonioscopy lens (four-mirror mini-gonioscopic laser lens; Ocular Instruments, Inc., Bellevue, WA) was used for the treatment, because its diameter flange was found convenient for the small palpebral fissure of cats. The laser energy was increased step by step until slight blanching of the treated tissue was obtained or formation of a small gas bubble occurred. When formation of a larger bubble was noted, the energy was slightly decreased. Depending on the degree of pigmentation of the cat’s anterior chamber angle (which varied over a certain spectrum from animal to animal), energy levels had to be titrated with all lasers until the desired tissue reaction occurred.

The mean number of shots applied per cat was 41 ± 12 in the Ti:Sapph laser group, 42 ± 2 in the alexandrite laser group, and 54 ± 14 in the argon laser group. The average energy applied per laser shot was 26, 14.5, 12.1, 9.8, and 16.4 mJ, respectively, for the eyes in the Ti:Sapph laser group; 17.2, 25, 27, 39.7, and 43.2 mJ, respectively, for the eyes in the alexandrite laser group, and 78, 57.5, 88.5, 89.8, and 89.6 mJ, respectively, for the eyes in the argon laser group. The trabecular meshwork was treated over 180°, leaving the second half of the chamber angle untreated and thus serving as a control. The target tissue for the treatment was the mid-portion of the trabecular meshwork. Treatments and both preoperative and postoperative slit-lamp examinations were video recorded and digital pictures were taken for later analysis. No systemic or topical anti-inflammatory therapy was administered.

The animal intended for sham treatment was anesthetized and positioned in front of the laser slit lamp, and the chamber angle of the left eye was inspected using a goniroscope lens similar to that used for the treated cats and for the same time duration, but no laser shots were applied. Preoperative and postoperative examinations identical to those for the other animals were performed.

Postoperative Examination

Biomicroscopy, goniscopy, and tonometry of the treated eyes were performed 1 hour postoperatively, at postoperative days 1 and 7, and weekly thereafter, always at the same time of the day. Two cats in each group were killed after 4 weeks, whereas three cats in each group were killed at 12 weeks postoperatively. The cat with sham trabeculectomy underwent the same postoperative management and was observed for 12 weeks.

Statistical Evaluation

The relative change of the treated eye relating to the untreated eye was expressed in terms of percentages (i.e., [treated - untreated]/untreated *100%). A value of 0 means that the IOP equals the untreated fellow eye. A value of 100 means that the IOP doubled (relative to the untreated fellow eye). For the comparisons of the different laser types at certain time points, an analysis of variance with post-hoc tests (LSD test) was performed (between-group comparisons). For the comparisons of a certain laser over time (i.e., preoperatively vs postoperatively), one-sided dependent t tests were applied (within-group comparisons). A categorized whisker plot with approximately 95% confidence
intervals illustrates the course of the relative changes over time. All statistical analyses were performed with STATISTICA 5.5 software (StatSoft Inc., Tulsa, OK). A P value of less than .05 was considered statistically significant.

Tissue Preparation

At the end of the observation period, the cats were killed by an intracardial injection of a lethal dose of pentobarbital and phenytoin (Eutrasol; Diamond Animal Heath, Inc., Des Moines, IA). The eyes were enucleated and the whole eyes were fixed in formalin 10%. An injection of 0.1 mL of formalin was also made into the anterior chamber. The cornea was cut circularly 2 mm parallel to the limbus and removed. All eyes were then cut at the equator, the posterior segments including vitreous and lens were carefully removed, and the remaining tissue was cut meridionally in four equal parts. This was performed in a way that all irradiated areas were contained entirely in two sections, with the two untreated sections serving as controls.

One treated and one untreated section of each eye were fixed for scanning electron microscopy in a mixture of paraformaldehyde 2% and glutaraldehyde 2.5% in Millonig’s phosphate buffer. To counteract closure of the angle structures in those specimens during the following dehydration process, the pupillary edge of the iris was gently bent downward and fixed to the inner wall of the sclera with interrupted sutures (Fig. 1). Greatest care was taken not to damage the angle architecture. The remaining sections (1 treated, 1 untreated) were kept in formalin 10% for light microscopy.

Light Microscopy

One treated and one untreated section of those eyes enucleated after a 4-week follow-up were further processed for histologic evaluation. To get sections from the actual laser spots, an extensive series of 5-μm cuts was obtained and consequently stained with hematoxylin–eosin, periodic acid-Schiff, and Masson trichrome techniques.

Scanning Electron Microscopy

After their initial fixation in 2% paraformaldehyde and 2.5% glutaraldehyde, the specimens intended for scanning electron microscopy were rinsed in Millonig’s phosphate buffer, postfixed in buffered osmium tetroxide (1%, 2 hours), rinsed in phosphate buffer, dehydrated in a graded series of ethanol, and critical point dried using carbon dioxide. The dried samples were mounted onto specimen stubs, gold coated with a Hummer V coater (Technics, Alexandria, VA), and observed with a JSM-35 scanning electron microscope (JEOL Ltd., Tokyo, Japan) at an acceleration voltage of 25 kV. Photographs of each sample were taken at a standard series of magnifications (×18, ×55, ×150, ×1,000, and ×6,000).

RESULTS

Clinical Observations

Following near-infrared laser trabeculoplasty, a minor inflammatory reaction (cells and flare) could be observed within the anterior chamber, which subsided within the first week. At postoperative day 7, the anterior chambers were completely quiet in all of the animals. Argon laser trabeculoplasty showed less inflammatory reaction; the anterior chamber was normal and quiet 1 day after laser treatment and also thereafter. Both near-infrared lasers created depigmentation in the area of the treated trabecular meshwork, which could be easily detected postoperatively by gonioscopy. With time, depigmentation of those laser spots increased in size, some reaching confluence (Figs. 2A-D). Eyes treated with argon laser displayed slight blanching of the treated tissue in the early postoperative phase only (Figs. 2E and F). Very slight focal blood staining in the chamber angle could be observed in some animals right after Ti:Saph laser trabeculoplasty (4 of 5 cats treated), as well as after alexandrite laser treatment (4 of 5 cats treated). The blood remnants were gradually
absorbed and not further detectable at postoperative day 14.

Formation of synechia was not observed following treatment with any of the lasers tested. Neither cornea nor iris displayed any changes due to the laser treatment. No cataract formation was observed during the follow-up time and the posterior segment appeared normal at all time points. None of the animals showed any signs of pain or loss of weight or fur.

**IOP Measurements**

The IOP at 1 hour, 1 day, and 1 week postoperatively was remarkably lower, irrespectively of the laser source used (Fig. 3). Mean IOP reduction after 1 week was -21.1% for the eyes treated with Ti:Sapph laser, -16.1% for the eyes treated with alexandrite laser, and -18.0% for the eyes treated with argon laser. After 2 weeks, statistically significant IOP reduction was found for the Ti:Sapph laser group (-16.2%) and for the argon
laser group (-18.0%), but not for the alexandrite laser group. Three weeks following treatment, a statistically significant IOP reduction was found in the alexandrite laser group only (-10.2%), whereas at the 4-week follow-up a statistically significant difference to the preoperative values could still be measured in the Ti:Sapphire laser group (-10.7%). An overview of all IOP changes over time (% difference to fellow eye) is given in Table 2. Comparing the different lasers at certain time points, the argon laser group had statistically significant less IOP reduction at the 3-week follow-up than the Ti:Sapphire laser group ($P = .031$) or the alexandrite laser group ($P = .039$). The cat receiving sham treatment showed no significant IOP change (Table 2).

**Light Microscopy**

Untreated sections displayed a chamber angle with trabecular beams originating from the internal surface of the sclera on one side and attached to the iris root and to the base of the ciliary body on the other. The collagenous core of the trabecular beams was surrounded by flattened endothelial cells. The trabecular meshwork was adjacent to an aqueous plexus and a band of cells containing pigment, which continued into the deep stromal layers of the cornea anteriorly and into the uveal tract posteriorly. Histologic evaluation of sections treated with argon laser showed focal disorganization of collagen fibers of the inner portion of the sclera adjacent to the trabecular meshwork with signs of mild scarring (Fig. 4A). Those changes could not be found after near-infrared laser treatment, although a focal decrease in pigment density could be observed underneath the treated trabecular meshwork (Fig. 4B).

**Scanning Electron Microscopy**

**4 Weeks Postoperatively.** All untreated sections displayed an unaltered trabecular meshwork with intact trabecular beams and a complete coverage with trabecular endothelial cells. In sections treated with argon laser, some of the laser impact sites could still be easily identified, with evidence of coagulative damage. They presented with disrupted, shrunken, and distorted trabecular beams (Fig. 5A). Higher magnification of those areas displayed that several trabecular beams had lost their endothelial coverage, with exposure of their collagenous core (Fig. 5B). In contrast, the architecture of the trabecular meshwork treated with either of the near-infrared lasers remained unchanged compared to
the untreated sections, and there was no evidence of mechanical damage. The laser impact sites could not be detected. Higher magnification displayed an intact endothelial covering with bleb-like extensions of the cell surface.

12 Weeks Postoperatively. Laser impact sites could not be identified in any of the eyes, irrespective of the laser source used (Fig. 5C). All trabecular beams were completely covered by endothelial cells. High magnification (×6,000) displayed fine microvilli-like extensions of the endothelial cell surface (Fig. 5D).

DISCUSSION

Cats have been used repeatedly for evaluating glaucoma surgery. The predominantly used animal model for experimental morphologic studies on laser trabeculoplasty so far has been the monkey, because its trabecular meshwork morphology is fairly similar to that of the human. However, argon laser trabeculoplasty has been shown to create scarring, an increase in IOP, and occasional glaucoma in that species, especially when higher energy levels were applied. Because we intended to also measure the IOP response to near-infrared laser application, we decided to use another animal model.

The anterior segment of the cat eye closely resembles the human eye, having a deep anterior chamber and a wide iridocorneal angle. This allows for easy access to the trabecular meshwork, making this species a potential model for laser trabeculoplasty. Experimental laser trabeculoplasty in cats has been performed previously. Ticho described destruction of pectinate ligaments and necrosis of the iris within the first week following argon laser trabeculoplasty, as well as scarring over the burned area after 3 months. This kind of iris damage was not observed in our study. However, the energy levels applied by Ticho per spot (up to 3000 mW) were far beyond those used in our study.

When interpreting the morphologic and histologic findings of our study, one has to carefully consider the anatomic differences of the chamber angle and also the slightly different pigment distribution within the trabecular meshwork between cats and humans (and also non-human primates). The cat's trabecular meshwork is situated predominantly in the uveal area in contrast to the human, where the trabecular meshwork has a substantial corneoscleral part. The trabecular beams are also more delicate and more uniformly oriented in the uveocorneal direction and the meshwork is much looser. Whereas pigment granules can be found everywhere in the trabecular endothelial cells in the human eye, only the pectinate ligaments—as sporadic extensions of the iris—contain significant amounts of melanin in cats. The trabecular meshwork itself is lacking any pigment and appears mainly transparent on gonioscopy. Thus, the layer of melanin-containing
Figure 5. Scanning electron microscopy. (A) Trabecular meshwork 1 month after argon laser treatment (500 mW). Laser impact site indicated with arrow (original magnification, ×150). (B) Trabecular beam lacking endothelial coverage and collagenous core of beam exposed (original magnification, ×6,000). (C) Trabecular meshwork 3 months after titanium:sapphire laser treatment (28 mJ) with no obvious damage to beam architecture detectable (original magnification, ×150). (D) Trabecular beam with unaltered endothelial coverage showing fine extensions of the cell surface visible (original magnification, ×6,000).

cells underneath appears as pigmented band and can be studied in its details (Figs. 1 and 2).

The process of focal depigmentation that could be detected following near-infrared laser trabeculoplasty in our study was therefore taking place underneath the trabecular meshwork. It can be assumed that the applied near-infrared laser radiation was selectively absorbed by the melanin pigment of this deep layer, leading to heat-induced damage of those pigment-containing cells. The released pigment was consequently removed by macrophages, which could serve as an explaination for the increasing depigmentation with time in the area of the laser spots. On the other hand, the argon laser resulted in a more superficial and unspecific coagulative damage and shrinkage to the collagen (Fig. 5A), which is in accordance with scanning electron microscopy studies on human eyes.29–32 Our findings indicate that the tested near-infrared lasers—emitting at a longer wavelength (λ =790 nm) compared to argon laser (λ = 488+514 nm) and also to Q-switched Nd:YAG laser (λ = 532 nm)—might actually penetrate deeper into the tissue, selectively altering pigment-containing cells. The unpigmented trabecular meshwork in front of it was found without any thermal or structural damage (Fig. 5C).

Also, the cat's aqueous outflow system is not strictly comparable to the human anatomy. Cats lack Schlemm's canal. Aqueous humor is collected from the trabecular meshwork by an aqueous plexus and drainage canals instead (Fig. 4A), entering the sclera at right angles and leading into wide aqueous veins.25 This might also explain the high rate of bleeding observed after the near-infrared laser treatment (which could not be observed after argon laser treatment), because the "deeper" effect within the trabecular meshwork may induce a temporary reflux of blood from the collecting veins. Hemorrhage of the trabecular meshwork as a complication of argon laser trabeculoplasty was seldom described,33 and could not be observed in our cats treated with argon laser. The mild iritis occurring in our study immediately after treatment could be explained by the "deeper" impact of the near-infrared lasers. A breakdown of the blood–aqueous barrier, as suggested by Feller and Weinreb, might be responsible for that transient phenomenon.34

A remarkable IOP reduction could be measured shortly after the laser procedure in our study. IOP
spikes, as described for human patients with glaucoma after argon laser trabeculoplasty within the first postoperative hour, did not occur. However, the IOP reduction in our animal study was not permanent and diminished within several weeks. The effect of the near-infrared lasers lasted a little longer compared to the argon laser. The IOP finally returned to preoperative values at 6 weeks within the Ti:Sapphire laser group, at 4 weeks within the alexandrite laser group, and at 3 weeks within the argon laser group. However, the number of animals per laser group observed for the complete observation period of 12 weeks was relatively low (n = 3), which affected the statistical evaluation.

Nevertheless, our findings are in sharp contrast to argon laser trabeculoplasty in human patients with glaucoma, where the hypotensive effect lasts up to years, and also to a prototype of a Ti:Sapphire laser, where a prolonged IOP reduction could be achieved for at least 1 year in a human pilot study. It seems that a prolonged IOP reduction is difficult to achieve in our specific animal model; furthermore, other methods are known to induce a permanent IOP reduction in human patients with glaucoma, but those had a temporary effect on cat eyes, even after cyclodestructive procedures. On the other hand, one has to consider that we treated healthy, non-glaucomatous eyes only. In human patients, the mean IOP change was also found to be less when pretreatment IOP before argon laser trabeculoplasty was below 20 mm Hg.

In this study, all calculations were based on IOP measurements using a pneumotonometer while the cats were under anesthesia. Thus, a higher reproducibility of the readings was found compared to a setting with the cats fully awake. Because the IOP is known to be altered by several anesthetic agents, including ketamine, the readings were taken after a certain time interval to limit its impact on the evidence of the measurements. By calculating the relative IOP change to the fellow untreated eye, the achieved results were found less dependent on that IOP fluctuation than calculation with absolute IOP values. In living cats, measurements using pneumotonometry were also found to be more reproducible than measurements using the Tono-Pen® (Tono-Pen XL; Medtronic Solan, Jacksonville, FL).

Although argon laser trabeculoplasty has now been performed for more than 20 years, the mechanism of IOP reduction induced by laser trabeculoplasty is still unknown and hypothetical. Several theories have been established and published in the literature. The mechanical theory notes that the heat-induced shrinkage of the trabecular beams widens intertrabecular spaces, facilitating the aqueous outflow. According to a cellular theory, the laser light induces a biological response of the trabecular endothelium, enhancing its macrophagic activity and consequently improving the outflow activity. The ability of trabecular meshwork cells in cats to perform phagocytosis has been shown previously. Because the architecture of the trabecular meshwork was maintained after irradiation with either near-infrared laser, the latter theory seems to be more likely to explain their effectiveness.

The applied energy per shot was selected according to the achieved tissue reaction, similar to the recommendations stated for argon laser trabeculoplasty treatment. Thus, we used energies for our near-infrared lasers that were more than 10 times higher than those currently used for selective laser trabeculoplasty. However, that difference can be explained by the decreased optical absorption by melanin at longer wavelengths. Accordingly, the threshold energy has to increase if lasers with a longer wavelength are used. It may well be that the minimal energy input necessary for permanent IOP reduction might actually be lower, which would reduce the total energy input and consequently the risk for any potential collateral damage.

Although the human glaucomatous eye may differ from the normal cat eye regarding response to laser treatment, valuable information on tissue–laser interaction of near-infrared laser trabeculoplasty has been gained from these experiments. Nevertheless, further in vitro studies seem mandatory prior to a human study to determine those treatment thresholds.

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