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A DISSERTATION
FOR THE DEGREE OF MASTER

**Effects of Transcorneal Iridal Photocoagulation
on *ex vivo* Canine Corneas
using an Endoscopic Semiconductor Diode Laser**

적출한 개의 각막에 내시경 다이오드 레이저를 이용한
경각막 홍채 광응고술이 미치는 영향

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using an Endoscopic Semiconductor Diode Laser**

**by
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**Supervised by
Professor Kangmoon Seo**

Thesis

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지도교수 서 강 문

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ABSTRACT

This study was to investigate the corneal changes after transcorneal iridal photocoagulation using an endoscopic semiconductor diode laser (endolaser) in *ex vivo* canine eyes. Seven normal canine eyes, which were enucleated following euthanasia for unrelated reasons, were used in this study. The cornea of each eye was divided into quadrants corresponding to four laser intensity groups. Each quadrant of the cornea was randomly irradiated using a semiconductor diode laser (OcuLight SLx; IRIS Medical Inc., Mountain View, CA, USA) and endolaser probe (Endo Optiks Inc., Little Silver, NJ, USA) with different laser intensities of 200 mW, 500

mW, 1000 mW, and 1500 mW, respectively. Three predetermined points of each quadrant were respectively irradiated for 5 min, for a total of 15 min per quadrant. Immediately after irradiating all four quadrants, the eye globe was placed into 4% paraformaldehyde for fixation. The corneal tissue samples were routinely processed for histological evaluation and stained with hematoxylin and eosin. No edematous lesions or shrinking were observed in the cornea in any of the four groups. Histologically, specific changes were not found in any of the four groups compared to normal corneal tissues. In conclusions, transcorneal iridal photocoagulation using the endolaser did not induce structural changes in the cornea despite the use of higher laser power than that used clinically (<1500 mW). The endolaser could be used for transcorneal iridal photocoagulation as well as endoscopic cyclophotocoagulation.

Key words: cornea, dogs, endolaser, iridal photocoagulation, transcorneal photocoagulation

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INTRODUCTION

In veterinary ophthalmology, laser treatment was first reported in the 1980s and various lasers have been used such as the CO₂, excimer, argon, neodymium:yttrium aluminum garnet (Nd:YAG), and diode lasers (Bartels, 2002; Gelatt and Gelatt, 2011). The most commonly used laser is the diode laser due to its relatively low cost, high convenience, and effective absorption by melanin (Bartels, 2002; Gelatt and Gelatt, 2011). It emits invisible infrared spectral energy at 810 nm and the light energy deeply penetrates the tissues until selectively absorbed by pigmented tissues (Bartels, 2002; Gelatt and Gelatt, 2011; Grahn and Peiffer, 2013). The absorbed energy is then converted into heat energy (Grahn and Peiffer, 2013). The heat-energy destroys the pigmented tissue and influences the surrounding tissues (Grahn and Peiffer, 2013). Theoretically, non-pigmented tissues such as clear cornea and sclera does not significantly absorb the laser (Chandler *et al.*, 2003; Gelatt and Gelatt, 2011). These tissues are affected by secondary heat energy rather than direct effect of the laser (Chandler *et al.*, 2003; Gelatt and Gelatt, 2011).

Laser delivery devices are instruments used to deliver laser energy safely and effectively from the laser source to the target tissue even within the eye (Gelatt and Gelatt, 2011). Many probes that connect to diode laser machines have been developed as laser delivery devices (Bartels, 2002; Gelatt and Gelatt, 2011). There are different types of laser probes depending on the pathway the laser is transmitted, such as the transscleral probe, endolaser probe, laser indirect ophthalmoscope (LIO), and operating microscope adapter (OMA) (Bedford, 1977; Cook and Wilkie, 1999; Gelatt and Gelatt, 2011; Gemensky-Metzler *et al.*, 2004; Hardman and Stanley,

2001). Among them, an endolaser probe was reported to be applied to canine and feline glaucoma in the 2000s (Badicu *et al.*, 2015). This is inserted into the eye via a limbal incision to deliver the laser directly to the ciliary body (Gelatt and Gelatt, 2011).

Unlike the transscleral probe and endolaser probe using diverging beams, LIO and OMA utilize converging beams (Bartels, 2002; Gelatt and Gelatt, 2011). The LIO is usually used with a 20D lens to produce the converging beams and accurately target the lesion. The OMA is operated with an operating microscope and a 175 mm objective lens to produce various sizes of converging beams; 0.3, 0.5, 0.8, 1.2, and 2.0 mm (Cook and Wilkie, 1999). For transcorneal procedures such as transpupillary retinopexy or transcorneal photocoagulation, the LIO and OMA are preferred over other probes that produce diverging beams (Cook and Wilkie, 1999; Gemensky-Metzler *et al.*, 2004). Because the converging system focuses the laser light on one point, iris lesions receive greater laser energy than the cornea during transcorneal iridal photocoagulation.

Recently, transcorneal photocoagulation using a diode laser with an LIO or OMA has been used as a non-invasive method for treating anterior uveal cysts or iris melanomas safely and effectively in dogs, cats, and horses (Cook and Wilkie, 1999; Gemensky-Metzler *et al.*, 2004). There are some complications such as corneal edema, dyscoria and iris hyperpigmentation, but cataract and glaucoma were not found in dogs (Cook and Wilkie, 1999). Another study investigating the effects of a diode laser with an OMA on the corneal endothelium during transcorneal iridal photocoagulation did not identify significant changes in the corneal endothelium by

specular microscopy or scanning electron microscopy (Chandler *et al.*, 2003). Despite increasing the power of the laser, there were no significant changes in the corneal thickness or endothelial cell density (Chandler *et al.*, 2003).

The endolaser probe with a diverging system used to spread a laser beam has not been tested for use as a laser delivery device for transcorneal treatment. Because diverging beams involve greater laser energy concentration near the probe tip, the cornea might be exposed to stronger energy than the iris during transcorneal photocoagulation. The purpose of this study was to investigate corneal changes after transcorneal iridal photocoagulation using an endoscopic semiconductor diode laser (endolaser) in *ex vivo* canine eyes.

MATERIALS AND METHODS

1. Preparation of enucleated canine eyes

Seven enucleated canine eyes were used for this research. The eyes were obtained from four beagle dogs without ocular diseases, and each animal was euthanized for reasons not related to this study. For maximum exposure of the iris during laser application, one drop of 2% pilocarpine eye drops (Isopto Carpine; Alcon, Fort Worth, TX, USA) was administered to each eye before euthanasia. The eyes were enucleated immediately after euthanasia by the routine palpebral technique. The enucleated eyes were stored in normal saline at $22\pm 2^{\circ}\text{C}$ and the experiments were performed within 10 h. This study was approved by the Institutional Animal Care and Use Committee of Seoul National University (SNU-180621-20).

2. Procedures

Each eye was fixed to a styrofoam plate with pins. All corneas were divided into four sections corresponding to four laser intensity groups, and each quadrant was marked with scleral sutures at the middle of quadrant circumference (Fig. 1). In addition, all eyes were marked using extra one knot at the 12 o'clock position of the globe to determine the exact direction of the eye (Fig. 1). Transcorneal iridal photocoagulation was performed using a semiconductor diode laser (OcuLight SLx; IRIS medical inc., Mountain View, CA, USA) and endolaser probe (Endo Optiks Inc., Little Silver, NJ, USA; beam size 500 μm , 19 g, straight tip) under an operating microscope (Leica F18; Leica Microsystems, Heerbrugg, Switzerland) by the same operator (YP). Each quadrant of the cornea was randomly irradiated with different laser intensities of 200 mW, 500 mW, 1000 mW, or 1500 mW. The laser of same intensity was applied at three sites per quadrant and each site was irradiated for 5 min for a total of 15 min per quadrant. The first site was at a distance of 2 mm vertical from the limbus adjacent to the marked suture at each quadrant. The other two sites were placed on both sides within 1 mm from the first indicated site, but did not overlap with the other sites (Fig. 1). During the irradiation, the laser probe was fixed using a stereotactic instrument so that the vertical distance from the laser probe tip to the cornea was maintained at 2 mm as measured by a castroviejo caliper (Fig. 2). Normal saline was irrigated every 15 s to keep the cornea moist during the laser applications. The other factors aside from

the laser power, such as duration and interval time, were the same in all seven eyes. The laser application duration was set at 9000 ms and the interval duration was set at 1000 ms.

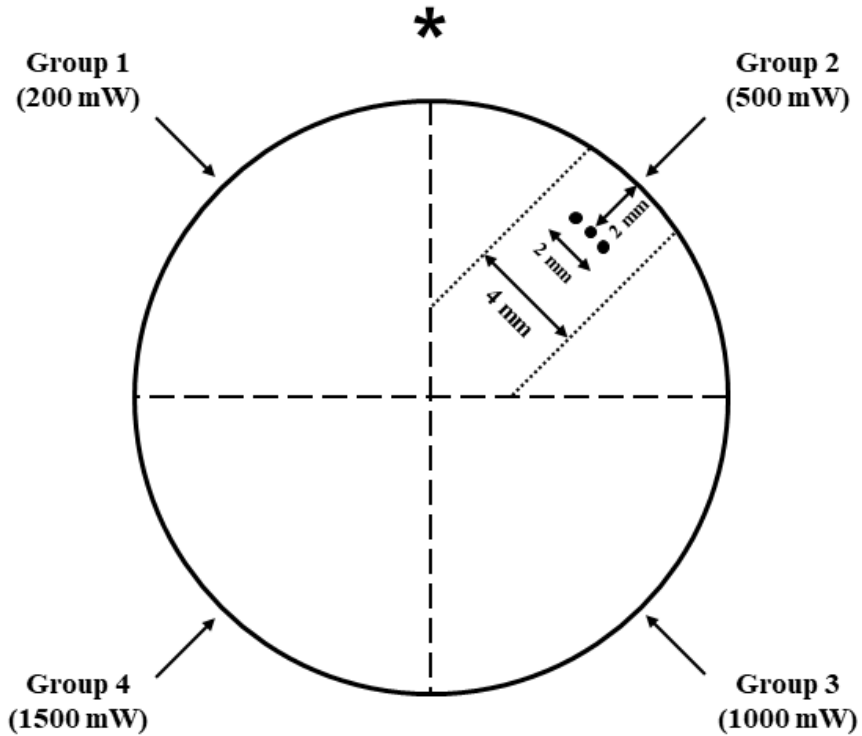


Fig. 1. Schematic diagrams of the cornea irradiated by the laser. The solid line circle indicates the limbus of the cornea, which is divided into four sections by the dashed line. An extra knot (asterisk) was placed at the 12 o'clock position to indicate the exact direction of the eye and each quadrant was marked with scleral sutures at the middle of the quadrant (arrow). The three sites irradiated by the laser were at a vertical distance of 2 mm from the limbus (three small circles) at a width of 2 mm. Each quadrant was cut to a width of 4 mm (dotted line) for histological evaluation.

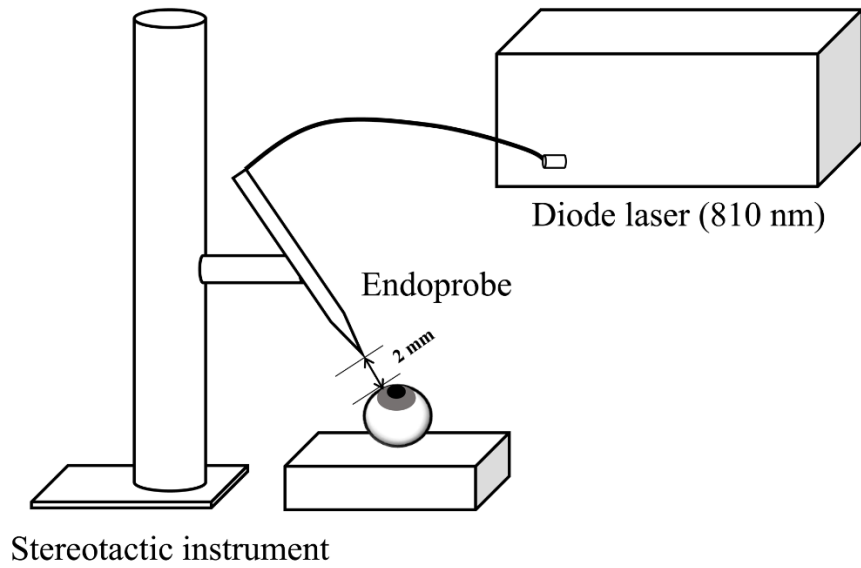


Fig. 2. Schematic diagram of the fixed laser probe using a stereotactic instrument. The vertical distance from the laser probe tip to the cornea was maintained at 2 mm.

3. Histological evaluation

Immediately after irradiating all four quadrants, the eyeball was dipped in 4% paraformaldehyde for fixation. The cornea was carefully separated from the fixed eyeball and cut into predetermined quarters using a microtome knife. Each quadrant was cut to a width of 4 mm centered on the pre-marked suture so as to include the irradiated area of 2 mm in width (Fig. 1). The dissected cornea tissues were embedded in paraffin wax to make tissue blocks and the blocks were trimmed before sectioning. The thickness of one section was 3 μm and two sections were taken every 20 μm in whole tissue block. These sections were stained with hematoxylin and eosin. The slides were scanned using a Panoramic MIDI scanner (3D-Histech, Budapest, Hungary) and reviewed with CaseViewer 2.0 software (3D-Histech, Budapest, Hungary). The irradiated area on the cornea, 2000 μm from the limbus, was measured by the program and both sides were widely observed in all sections (Fig. 3).

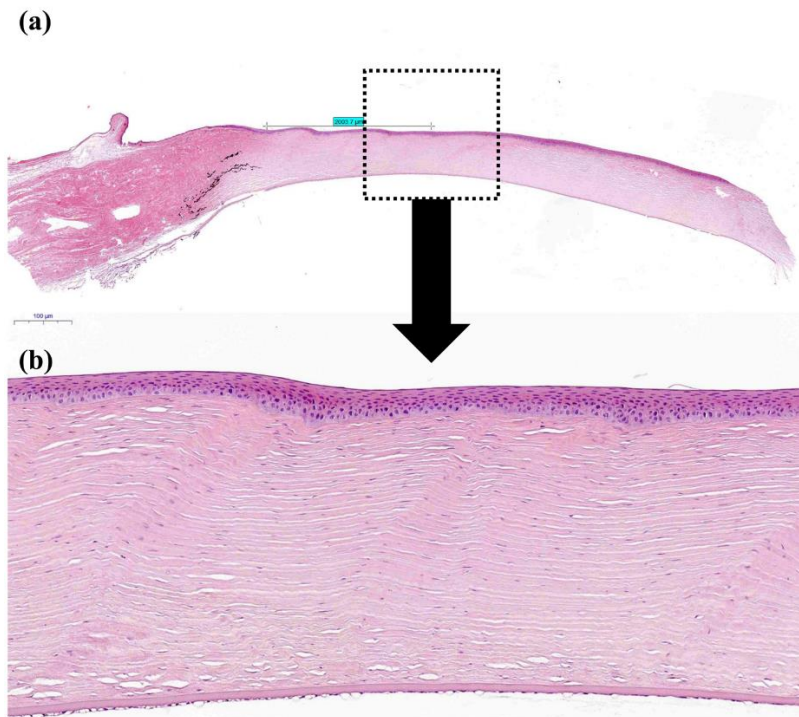


Fig. 3. Representative images of histological corneal tissue irradiated by the laser. (a) A distance of 2000 μm from the limbus was measured by the program. (b) No specific changes were observed in the cornea (Hematoxylin and eosin stain).

RESULTS

While irradiating the corneas under the surgical microscope, no gross changes, such as corneal edema and thermal shrinkage, were observed in any of the four groups. The most common gross changes were iris hyperpigmentation and dyscoria. At the laser powers of 200 mW, 500 mW, 1000 mW, and 1500 mW, iris hyperpigmentation was observed in 0, 2, 6, and 6 eyes, respectively (Fig. 4). Dyscoria was observed in 6 eyes when the cornea was irradiated at ≥ 1000 mW (Fig. 4) and pigment cell dispersion caused by iris burning was observed in 2 eyes at 1500 mW (Table 1).

On the irradiated areas of the corneas, specific histological changes were not observed in any of the four groups. Compared to normal cornea tissues, structural abnormalities of the cornea, such as edematous lesions, collagen coagulation, disarrangement of stromal fibers, or epithelial and endothelial cell damage, were not observed in any sections (Fig. 5).

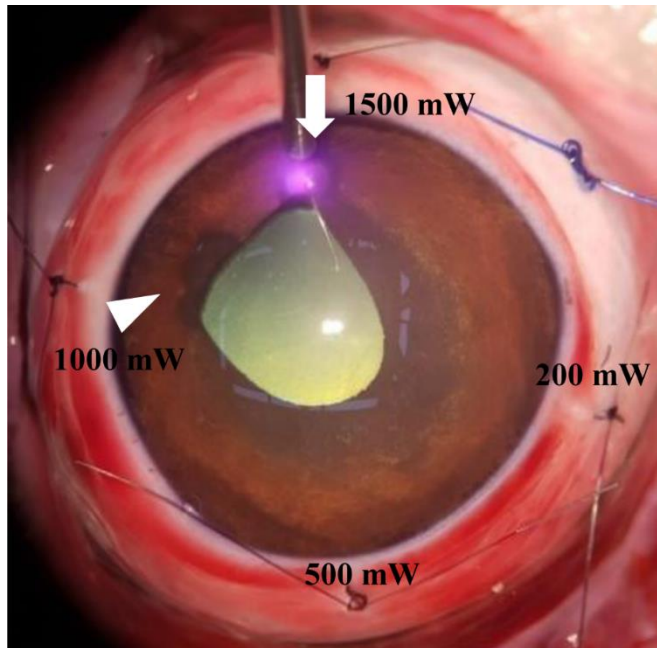


Fig. 4. Gross changes induced by laser irradiation. Iris hyperpigmentation (arrow head) and dyscoria (arrow) were observed at both 1000 mW and 1500 mW.

Table 1. Number of eyeballs with gross changes according to the laser power (four groups)

	Group 1 (200 mW, 5 min)	Group 2 (500 mW, 5 min)	Group 3 (1000 mW, 5 min)	Group 4 (1500 mW, 5 min)
Iris hyperpigmentation	0 / 7 eyes*	2 / 7 eyes	6 / 7 eyes	6 / 7 eyes
Dyscoria	0 / 7 eyes	0 / 7 eyes	6 / 7 eyes	6 / 7 eyes
Pigment cell dispersion	0 / 7 eyes	0 / 7 eyes	0 / 7 eyes	2 / 7 eyes

* Incidence number of eyes / total number of eyes

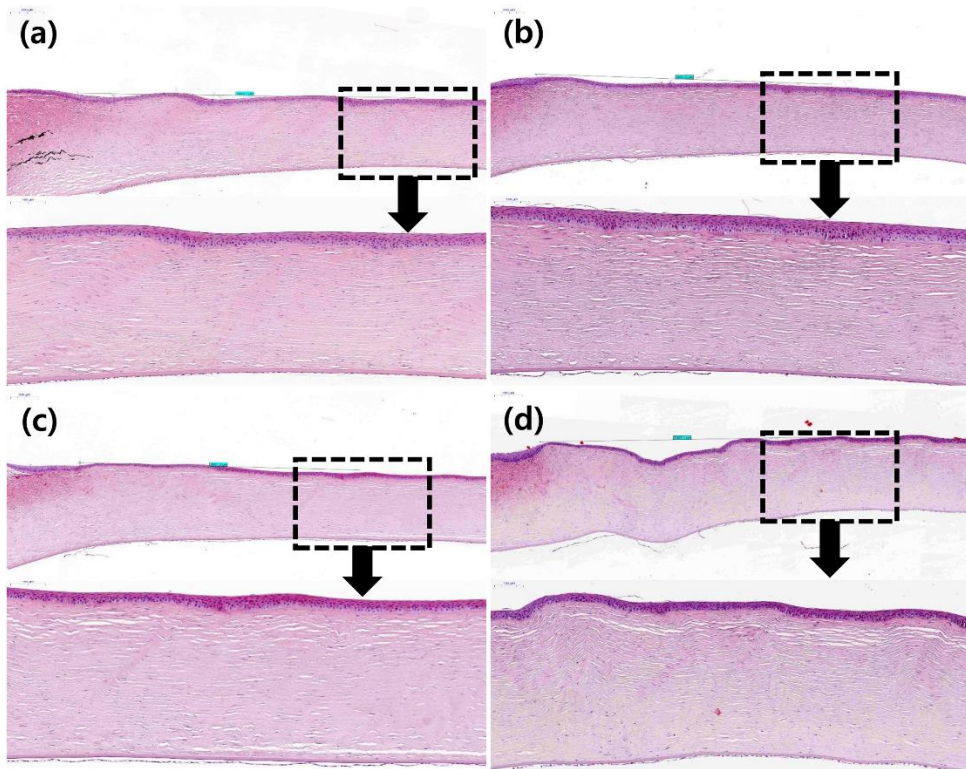


Fig. 5. Representative images of histological corneal tissue irradiated by the endolaser in four groups according to laser power. The laser of same intensity was applied at three sites and each site was irradiated for 5 min for a total of 15 min per quadrant. No specific changes were observed in the all section of corneas (Hematoxylin and eosin stain). (a) Group 1; 200 mW, (b) Group 2; 500 mW, (c) Group 3; 1000 mW, (d) Group 4; 1500 mW.

DISCUSSION

The diode laser has been applied to various ocular diseases (Bartels, 2002; Gelatt and Gelatt, 2011; Puliafito *et al.*, 1987). It is primarily used for glaucoma treatments such as transscleral or endoscopic cyclophotocoagulation (ECP) by destroying the ciliary body and thus reducing the production of aqueous humor in dogs, cats, and humans (Badicu *et al.*, 2015; Bras and Webb, 2009; Bras *et al.*, 2005; Gaasterland and Pollack, 1992; Harrington *et al.*, 2013; Lin, 2002). In addition, transpupillary or transscleral retinopexy using a diode laser has been performed to prevent or treat retinal detachment in humans and dogs (Bartels, 2002; Gelatt and Gelatt, 2011; Isola *et al.*, 2001; Kapran *et al.*, 2001; Spatola *et al.*, 2015). Recently, uveal diseases such as pigmented iris melanomas or uveal cysts have also been treated by diode laser therapy involving delivery of laser energy via the cornea without surgical excision (Bartels, 2002; Cook and Wilkie, 1999; Gelatt and Gelatt, 2011; Gemensky-Metzler *et al.*, 2004).

Uveal melanoma, or melanocytoma, is the most common intraocular tumor, which originates from the iris, ciliary body, and, rarely, the choroid in dogs (Bartels, 2002; Maggs *et al.*, 2018). It is locally invasive, but the metastatic rate is low in dogs (Gelatt *et al.*, 2013; Gemensky-Metzler *et al.*, 2004). Depending on the size and localization of the tumor, local excision with iridectomy, iridocyclectomy, enucleation, or laser photocoagulation have been applied as treatment methods (Bartels, 2002; Cook and Wilkie, 1999; Gelatt *et al.*, 2013). Uveal cysts are fluid-filled lesions derived from the posterior pigmented epithelial layer of the iris or ciliary body in dogs (Maggs *et al.*, 2018). They could be attached to the anterior uvea

or free-floating within the anterior chamber (Corcoran and Koch, 1993; Maggs *et al.*, 2018). In general, treatment is not necessary for dogs with uveal cysts because most cases are benign and have no clinical signs (Gelatt *et al.*, 2013; Maggs *et al.*, 2018). However, vision interference via obstruction of the pupil, development of glaucoma caused by iris displacement, or corneal edema by contact with the corneal endothelium occurs, cyst removal is required (Maggs *et al.*, 2018). Aspiration using a fine needle at the limbus or corneal incision for manual extraction could be applied as a treatment, and laser photocoagulation using an Nd:YAG or a diode laser can be considered to deflate the cysts as a non-invasive therapy (Maggs *et al.*, 2018).

Surgical interventions in uveal cysts and melanomas are invasive and could present the risk of infection, anterior uveitis, and hemorrhage (Gelatt and Gelatt, 2011; Gemensky-Metzler *et al.*, 2004; Maggs *et al.*, 2018). However, laser therapy using a diode laser could target the affected tissues without surgical incision and minimize complications (Gemensky-Metzler *et al.*, 2004; Maggs *et al.*, 2018).

In one study on transcorneal diode laser photocoagulation with an OMA or LIO for the treatment of iris melanoma in 23 dogs, only two dogs exhibited persistent corneal edema caused by collateral hyperthermia from the significantly elevated lesion (Cook and Wilkie, 1999). Other than corneal edema, only mild complications including iris hyperpigmentation, dyscoria, and pigment dispersion were observed (Cook and Wilkie, 1999). Postoperative uveitis was also decreased within 1 week (Cook and Wilkie, 1999). The laser power range used in these melanoma cases was 80 mW to 1000 mW and cumulative durations were 9 s to 14 min, 31 s (0:09 ~ 14:31)

(Cook and Wilkie, 1999). The laser was irradiated in a continuous mode and the laser power was adjusted according to the effect on the lesion (Cook and Wilkie, 1999).

In other study on transcorneal diode laser photocoagulation with an OMA or LIO for the treatment of iris cysts in 20 cases, cyst remnants and aqueous pigment dispersion were observed in all cases, but aqueous flare or discomfort was absent to minimal (Gemensky-Metzler *et al.*, 2004). The laser power range used in these cysts cases was 300 mW to 1200 mW and duration range was 200 ms to 2000 ms (Gemensky-Metzler *et al.*, 2004). Total energy range was 0.9 J to 285 J (Gemensky-Metzler *et al.*, 2004).

In this study, transcorneal iridal photocoagulation was performed using a semiconductor diode laser with an endolaser probe instead of an LIO or OMA. Laser powers used in this study was 200, 500, 1000 and 1500 mW and the cumulative duration was 5 min per site, for a total of 15 min per quadrant, so as to include a range of clinically used powers and durations for the treatment of iris cysts and melanomas. When using LIO or OMA in previous study, the maximum laser power for deflating uveal cysts was 1200 mW and the maximum cumulative time for irradiating melanoma was 14 min, 31 s (Cook and Wilkie, 1999; Gemensky-Metzler *et al.*, 2004). In the previous report, the recommended initial power and duration to deflate iris cysts in dogs was 1000 mW and 500 ms, respectively, and these parameters could be modulated depending on the cyst size, degree of pigmentation, and reactions during the procedure (Gemensky-Metzler *et al.*, 2004). Unlike iris cysts, tumors were better treated with emphasis on a hyperthermic response with

lower power and longer duration, such as in a continuous mode (Cook and Wilkie, 1999).

After laser irradiation, the observed gross changes such as dyscoria and iris hyperpigmentation were similar to those previously reported studies, and there were no specific gross changes in the cornea in any group (Cook and Wilkie, 1999; Gemensky-Metzler *et al.*, 2004). Pigment cell dispersion during iris burning was observed at 1500 mW in two eyes. These changes could be affected by the degree of underlying iris pigmentation and the laser power setting. When these factors are increased, complications like dyscoria, hyperpigmentation, or excessive burning could also increase. Therefore, the laser power is recommended to be less than 1000 mW and should be adjusted according to the degree of pigmentation and size of the lesion.

Tissues near the target lesions could be influenced by the heat energy generated at the target site by a diode laser (Grahm and Peiffer, 2013). In general, heat energy could induce thermal damage in the tissue and denature collagen by coagulation effects such as electrocautery (Grahm and Peiffer, 2013). In this study, for serial histological evaluation, the corneal tissues including the irradiated sites were observed every 20 μm and no specific changes such as coagulation effects in the epithelium, stroma, Descemet's membrane or endothelium were observed even at 1500 mW for 5 min, which were a higher laser power and cumulative duration than those used clinically.

Significant corneal damage in histological examination may not have been found for several reasons in this study. First, the distance from the targeted iris tissues to

the cornea may have been too great to be affected by collateral thermal energy because normal eyes without iris lesions were used. In clinical cases, when the iris lesion is significantly elevated, the cornea tends to be more exposed to collateral heat released from the irradiated lesion (Cook and Wilkie, 1999). Second, because the laser irradiated enucleated canine eyes, differences may exist between these eyes and living tissues, such as the level or rate of energy absorption and vital reactions in irradiated tissues. Also, in this study, the laser reaches the iris and induces iris changes such as iris hyperpigmentation and dyscoria, but it is difficult to compare how effective the endolaser is on the uveal cysts or melanoma in comparison to LIO or OMA, which treated iris lesions effectively in the previous study (Cook and Wilkie, 1999; Gemensky-Metzler *et al.*, 2004).

Further research is needed to investigate gross changes in the patients during irradiation and clinical signs after transcorneal iridal photocoagulation using an endolaser in dogs with uveal cysts or melanoma. A study involving the long-term follow-up of complications including corneal edema, iridal changes, uveitis, and endothelial damages evaluated by specular microscopy is also required prior to the clinical application of this method.

CONCLUSIONS

This study was designed to determine whether the endolaser used for ECP in glaucoma patients could also be used to treat uveal cysts or melanomas via transcorneal iridal photocoagulation. In normal enucleated canine eyes, no specific gross or histological changes were observed in the cornea despite the use of higher laser power (<1500 mW) and duration (5 min) than those clinically used. Based on these results, the endolaser could be used for transcorneal iridal photocoagulation as well as ECP, without corneal damages.

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국 문 초 록

적출한 개의 각막에 내시경 다이오드 레이저를 이용한 경각막 홍채 광응고술이 미치는 영향

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수의학과 임상수의학 전공

본 연구는 개의 적출한 눈에서 내시경 다이오드 레이저(endoscopic semiconductor diode laser; endolaser)를 이용하여 경각막 홍채 광응고술(transcorneal iridal photocoagulation) 후 각막의 조직학적 변화를 평가하고자 실시하였다.

다른 질병이 없는 정상적인 개의 안구 7개를 사용하였으며, 본

연구와는 관련이 없는 이유로 안락사를 한 직후 안구를 적출하였다. 각 안구의 각막은 사등분으로 나누어 각각 200 mW, 500 mW, 1000 mW, 1500 mW의 세기를 임의의 순서로 조사하였으며, 레이저 세기에 따라 4개의 군으로 구분하였다. 레이저 조사는 다이오드 레이저 기계와 내시경 레이저 장치를 이용하여 실시하였으며, 각 사분면에서 동일한 3개의 지점을 5분씩, 총 15분간 조사하였다. 모든 사분면을 조사한 후, 4% 파라-포름알데히드에 넣어 안구를 고정시키고, 조직학적 평가를 위한 일반적인 샘플 처리와 H&E 염색을 실시하였다.

4개의 군 모든 각막에서 부종, 수축 등의 육안적인 병변이나 정상 각막 조직과 비교하여 조직학적으로 유의미한 병변은 보이지 않았다.

본 연구 결과 내시경 다이오드 레이저를 이용한 경각막 홍채 광응고술 시 임상적으로 사용된 것보다 높은 레이저 세기(<1500mW)를 사용함에도 불구하고 각막의 구조적 변화는 관찰되지 않았다. 따라서 내시경 다이오드 레이저를 이용한 경각막 홍채 광응고술은 전안방의 홍채 낭종이나 홍채에서 발생한 흑색종을 가진 환자에서 각막의 손상 없이 치료할 수 있는 방법으로 고려될 수 있다.

주요어: 각막, 개, 다이오드 레이저, 홍채 광응고술, 경각막 광응고술

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