

# **Treatment Parameters for the Efficacy of Transscleral Cyclophotocoagulation in Rabbits Using a Diode Laser**

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**Purpose:** To determine parameters for the efficacy of transscleral cyclophotocoagulation (TSCPC) using a diode laser.

**Methods:** We performed TSCPC on 74 pigmented rabbits with different exposure powers and varying number of applications, followed by clinical observation and histological examination up to 24 weeks.

**Results:** Based on observation of the clinical course, the most favorable parameters were 600 mW and 36 or 48 applications, which did not cause severe complications and sufficiently lowered intraocular pressure (IOP). Histological examination revealed coagulation of the epitheliums and stroma of the ciliary body at 600 mW. The stroma of the ciliary body was severely damaged at 900 mW.

**Conclusions:** Transscleral cyclophotocoagulation at 600 mW with a larger number of applications than previously reported did not cause severe complications and effected greater and more lasting lowering of IOP than TSCPC with more intense coagulation and fewer applications. **Jpn J Ophthalmol 2000;44:205–213** © 2000 Japanese Ophthalmological Society

Key Words: Complications, histology, hypotony, intraocular pressure, rabbit eye.

# Introduction

Patients with refractory glaucoma, which is not responsive to medication and surgical procedures such as trabeculectomy, need to undergo cyclodestructive surgery. One of the conventional methods of cyclodestructive surgery is cyclocryotherapy. However, this method has certain disadvantages, such as severe postoperative inflammation and difficulty in predicting the level to which intraocular pressure (IOP) would be lowered after surgery. Recently, transscleral cyclophotocoagulation (TSCPC) using near-infrared lasers, such as Nd:YAG and diode lasers, has been performed, because of better permeability of light from these lasers through the sclera. The near-infrared diode laser is easy to handle because it is compact. Since the area and intensity of coagulation can be controlled more easily with TSCPC than with cyclocryotherapy, a more precise prediction of the lowering of the IOP level and reduction of complications can be expected with TSCPC.<sup>1</sup>

In TSCPC with the diode laser, two different methods are used: the contact method, in which the tip of the fiber contacts the sclera, and the noncontact method. Almost all clinical studies have used the contact method, which makes it easier to keep exposure power constant. In these studies, however, treatment parameters such as exposure power, number of applications, and extent of laser exposure have varied widely, and there was great variation in the therapeutic effects obtained and in the incidence of adverse reactions. With methods using relatively high exposure energy (3-6.8 J) and 20 or fewer applications, lowering of IOP could be achieved, but development of complications such as choroidal effusion, vitreous hemorrhage, hyphema, and hypotony occurred.<sup>2-4</sup> With methods using 2.3 J and 40 applications (lower exposure energy and larger number of applications) reported by Bloom et al,<sup>5</sup> the percentage of success was low but there were no severe complications. Thus, the treatment parameters for TSCPC have not yet been clarified, and it is necessary to determine with precision the parameters in

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which TSCPC can achieve effective lowering of IOP without severe complications.

In this study, we histologically examined the degree of ciliary body destruction in pigmented rabbits after TSCPC with a near-infrared diode laser at different power levels, and the relationship between treatment parameters including exposure power and number of applications and operative results, such as lowering of IOP and incidence of complications.

# **Materials and Methods**

#### Animals

We used 74 pigmented rabbits. Seventy-two animals were clinically followed up after surgery. Two other animals were enucleated immediately after surgery. All operative procedures were performed under general anesthesia by intramuscular injection of ketamine hydrochloride (20 mg/kg) and xyladine (2 mg/kg). Measurement of IOP after surgery was performed under local anesthesia by instillation of 4% oxybuprocaine hydrochloride. This experiment was performed in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research.

## Transscleral Cyclophotocoagulation

We used a near-infrared diode laser (wavelength 804 nm, Nidek DC-3000, Gamagori) for TSCPC. The laser beam was guided through crystal fibers (400  $\mu$ m in diameter), and the diameter of the tip of the fiber was 400  $\mu$ m (mounted with metal frame 400- $\mu$ m thick). The front edge of the cycloprobe was adjusted at the limbus and perpendicularly to the sclera, so that the center of the fiber tip was located at 600  $\mu$ m from the limbus. Irradiation was performed with slight compression by the fiber against the sclera. The duration of exposure was set at 2.0 seconds. Exposure power was measured by a power meter (Coherent Fieldmaster; Coherent, Palo Alto, CA, USA) at the edge of the fiber.

## Clinical Observation

Transscleral cyclophotocoagulation was performed in 72 eyes of 72 animals at 300, 600, 900, 1200, 1800, or 2800 mW, with 12, 24, 36, 48, 60, or 72 applications circumferentially over all quadrants (360°). The contralateral eyes were considered nontreated controls. Measurement of IOP, slit-lamp microscopy, and indirect ophthalmoscopy were performed before surgery and during the period of observation. Table 1 shows treatment parameters, periods of observation, and the number of tested eyes. Animals that developed phthisis bulbi during the period of observation were excluded from the estimation of IOP. We used a pneumatonograph (PTG; applanation pneumatonograph; Alcon, Fort Worth, TX, USA) for measurement of IOP. In order to exclude differences between right and left eyes, the change in IOP was calculated using the following formula:

Change in IOP = (postoperative IOP – postoperative control IOP) – (preoperative IOP – preoperative control IOP)

For each data point, the mean and standard deviation of the change in IOP were calculated. The IOP data were analyzed using the paired Student's *t*-test.

## Histological Examination

Transscleral cyclophotocoagulation was performed 8 times at 300, 600, 900, 1200, 1800, or 2800 mW, respectively, in 4 eyes of 2 animals. Eyeballs were enucleated immediately after surgery. Among animals subjected to clinical observation, 22 animals that underwent TSCPC with 36 applications at 600 or 900 mW underwent enucleation of eyes under general anesthesia at the end of clinical observation. These animals were sacrificed by an intravenous overdose of pentobarbital sodium immediately after enucleation. Treatment parameters, periods of observation, and number of observed eyes are shown in Table 2. Enucleated eyeballs were bisected along the equator line in a solution of 2.5% glutaraldehyde

**Table 1.** Clinical Observation (n = 72)

Exposure Power (mW)	Number of Applications of TSCPC*	Period of Observation (Weeks)	Number of Tested Eyes	
300	12	5	4	
600	12	5	4	
600	36	1–24	11	
600	48	24	5	
600	60	24	5	
600	72	24	7	
900	12	8	4	
900	24	3	4	
900	36	1–24	11	
900	48	24	5	
1200	12	5	4	
1800	12	5	4	
2800	12	5	4	

\*TSCPC: transscleral cyclophotocoagulation.

Exposure	Time After Surgery (Weeks)					
Power (mW)	0*	1	3	8	24	
300	4	_	_	_	_	
600	4	2	2	2	5	
900	4	2	2	2	5	
1200	4	-	-	-	_	
1800	4	_	_	_	_	
2800	4	-	-	-	-	

Table 2. Number of Animals Examined Histologically (n = 26)

\*Immediately after surgery.

and 2.0% paraformaldehyde (0.1 M cacodylate acid buffer solution), and fixed in this solution, embedded in paraffin, cut in 4-µm-thick sections, stained with hematoxylin and eosin, and examined with a microscope.

#### Results

#### Clinical Observation

Lowering of IOP. At 3 days after surgery, the IOP in all the operated eyes was reduced under all treatment conditions tested.

Twelve applications at 300 mW induced statistically significant (P < .05) lowering of IOP continuously for 1 week after surgery (Figure 1).

At 600 mW, a larger number of applications induced greater and more long-lasting lowering of IOP (Figure 2). The periods over which statistically significant lowering of IOP continued were 5, 12, 8, 20, and 24 weeks after TSCPC with 12, 36, 48, 60, and 72 applications, respectively.

At 900 mW, a larger number of applications induced greater lowering of IOP (Figure 3). The periods over which statistically significant lowering of IOP continued were 3 and 6 weeks after TSCPC with 12 and 36 applications, respectively. For TSCPC with 24 and 48 applications, the results varied widely, and no statistically significant lowering of IOP was observed, but there was a tendency for a larger number of applications to result in a longer period of lowering of IOP.

The periods over which statistically significant lowering of IOP continued were 2, 1, and 8 weeks after TSCPC with 12 applications at 1200, 1800, and 2800 mW, respectively (Figure 1).

Table 3 summarizes the mean values of the maximum decrease in IOP, the periods of maximum lowering of IOP, the mean value of IOP at the end of observation, and the periods over which statistically significant lowering of IOP was achieved.

Complications. Transscleral cyclophotocoagulation at 300 mW induced only slight infiltration of inflammatory cells in the anterior chamber. After TSCPC at 600 or 900 mW, a larger number of applications caused more intense inflammatory reaction in the anterior chamber. After TSCPC at 1800 or 2800 mW, severe inflammatory reaction in the ante-

Figure 1. Time course of changes in intraocular pressure (IOP) after transscleral cyclophotocoagulation. Exposure power: 300 mW (**■**), 600 mW (**●**), 900 mW (**▲**), 1200 mW (□), 1800 mW (○), 2800  $mW(\diamondsuit)$ , 12 applications. Change in IOP = (postoperative IOP - postoperative control IOP) - (preoperative IOP – preoperative control IOP). \*P < .05.



Time after surgery (days)



Figure 2. Time course of changes in intraocular pressure (IOP) after transscleral cyclophotocoagulation. Exposure power: 600 mW, 12 applications ( $\blacksquare$ ), 36 applications ( $\bigcirc$ ), 48 applications (▲), 60 applications ( $\square$ ), 72 applications ( $\bigcirc$ ). Change in IOP = (postoperative IOP – postoperative control IOP) – (preoperative IOP – preoperative control IOP). \**P* < .05.

rior chamber was common even with 12 applications. Inflammatory cells in the anterior chamber disappeared within 5 weeks after surgery in all cases without special treatment. Posterior synechia was observed after TSCPC with more than 36 applications at 600 mW, with more than 48 applications at 900 mW, and with 12 applications at more than 1800 mW (Table 4).

As shown in Table 4, vitreous hemorrhage was ob-

served after TSCPC at 900 mW. Cataract and *rubeosis iridis* were also observed in 2 of 4 eyes after TSCPC with 12 applications at 2800 mW. Although it was difficult to examine the fundus in these 2 eyes with cataract, vitreous hemorrhage was observed in the other 2 eyes without cataract after TSCPC with 12 applications at 2800 mW. Phthisis bulbi developed in 3 of 5 eyes and 5 of 7 eyes with 60 and 72 applications at 600 mW, respectively. Phthisis bulbi de-



Figure 3. Time course of changes in intraocular pressure (IOP) after transscleral cyclophotocoagulation. Exposure power: 900 mW, 12 applications ( $\blacksquare$ ), 24 applications ( $\bigcirc$ ), 36 applications ( $\blacktriangle$ ), 48 applications ( $\square$ ). Change in IOP = (postoperative IOP – postoperative control IOP) – (preoperative IOP – preoperative control IOP). \*P < .05.

Treatment Parameters				IOP at End	
Exposure Power (mW)	Number of Applications of TSCPC	Maximum Decrease in IOP and Period		of Observation	Period Over Which Statistically Significant ( $P < 05$ )
		Mean ± SD (mm Hg)		(mm Hg)	Decrease in IOP Occurred
300	12	$-7.3 \pm 3.8$	1 week	$-1.7 \pm 3.1$	3 days–1 week
600	12	$-7.5 \pm 1.6$	1 week	$-4.7\pm0.9$	3 days–5 weeks
600	36	$-15.2 \pm 4.5$	3 days	$-2.1 \pm 2.7$	3 days-8 weeks, 12 weeks
600	48	$-15.8 \pm 3.2$	3 days	$-5.5 \pm 5.9$	3 days–8 weeks
600	60	$-13.0 \pm 4.2$	2 weeks	$-0.5 \pm 2.1$	3 weeks, 10–20 weeks
600	72	$-18.5 \pm 14.9$	16 weeks	$-15.0 \pm 1.4$	4 weeks, 24 weeks
900	12	$-5.6 \pm 3.8$	1 week	$-2.1 \pm 3.2$	3 days–1 week, 3 weeks
900	24	$-4.4 \pm 4.2$	2 weeks	$-2.5 \pm 3.3$	None
900	36	$-13.3 \pm 7.0$	2 weeks	$-1.0 \pm 3.0$	3 days, 2 weeks, 5 weeks
900	48	-15.0	6 weeks	-9.0	None
1200	12	$-7.1 \pm 1.9$	1 week	$-1.6 \pm 1.3$	3 days–2 weeks
1800	12	$-4.1 \pm 2.7$	3 days	$-0.8 \pm 1.8$	3 days–1 week
2800	12	$-9.5 \pm 2.2$	5 weeks	$-9.2 \pm 1.4$	3 days–8 weeks

 Table 3. Maximum Decrease in Intraocular Pressure (IOP) and IOP at End of Observation with Various

 Treatment Parameters

veloped in 2 of 11 eyes and 4 of 5 eyes with 36 and 48 applications at 900 mW, respectively (Table 4).

#### Histological Examination

**Immediately after surgery.** Macroscopically, slight grayish-white coagulation spots (mild coagulation) were observed in 2 of the 8 lesions at 300 mW, and in 6 of the 8 lesions at 600 mW (Figure 4A). Clearly demarcated white, round coagulation spots (moderate coagulation) were observed in all irradiated regions at 900 mW (Figure 4B). Destruction of ciliary processes (severe coagulation) was observed after TSCPC at 1200 mW or higher power (Figure 4C).

Microscopically, coagulation necrosis of the ciliary epitheliums was observed after TSCPC at 300–600 mW. At these exposure levels, the stroma of the ciliary body became edematous, collagen fibers could not be observed clearly, and melanocytes in the stroma were destroyed. The sclera in coagulated regions became eosinophilic and its fibrous structure was destroyed. The damaged area was 0.79 mm<sup>2</sup> (Figure 5A). After TSCPC at 900 mW, the ciliary epitheliums was detached from the shrunken stroma but its continuity was preserved. In the stroma, edematous change was widespread next to the shrunken area. The damaged area was 1.63 mm<sup>2</sup> (Figure 5B). At 1200 mW, the epithelium was disrupted and the

Table 4. Complications

Exposure Power (mW)	Number of Applications	Mild Iritis	Moderate or Severe Iritis	Posterior Synechia	Vitreous Hemorrhage	Cataract	Rubeosis	Phthisis
300	12	1/4	0	0	0	0	0	0
600	12	2/4	0	0	0	0	0	0
600	36	7/11	4/11	2/11	0	0	0	0
600	48	2/5	3/5	1/5	0	0	0	0
600	60	0/2	2/2	2/2	0	0	0	3/5
600	72	0/2	2/2	2/2	0	0	0	5/7
900	12	3/4	1/4	0	2/4	0	0	0
900	24	4/4	0	0	0	0	0	0
900	36	7/9	2/9	0	0	0	0	2/11
900	48	0/1	1/1	1/1	0	0	0	4/5
1200	12	4/4	0	0	1/4	0	0	0
1800	12	1/4	3/4	2/4	3/4	0	0	0
2800	12	0/4	4/4	4/4	2/2*	2/4	2/4	0

\*Fundus examination was impossible in 2 eyes with cataract.



**Figure 4.** Macroscopic photographs immediately after transscleral cyclophotocoagulation. (A) 600 mW. Slight gray-white coagulation spot at ciliary body (mild coagulation). Damaged area is 500  $\mu$ m in diameter. (B) 900 mW. Clear white round coagulation spots at ciliary body (moderate coagulation). Damaged area is 720  $\mu$ m in diameter. (C) 1200 mW. Coagulation spots with destruction of ciliary process (severe coagulation). Damaged area is 860  $\mu$ m in diameter.

stroma of the ciliary body destroyed. In the stroma, pseudocyst formation was evident. Remnants of cellular components were observed within the posterior chamber. The damaged area was 2.32 mm<sup>2</sup> (Figure 5C). Perforation of the sclera was not observed after TSCPC at any level of exposure power.

**One week after surgery.** At 600 mW, spindleshaped cells were stratified at the surface of the lesion. The stroma of the lesion was atrophic and thinned. Accumulation of round pigment-laden cells was observed in the stroma. At 900 mW, spindleshaped cells were stratified. Accumulation of round pigment-laden cells was more in evidence than in coagulated regions at 600 mW, and hemorrhage and widespread edematous change were observed in the stroma. Exudative change was observed in the posterior chamber.

**Three to eight weeks after surgery.** At 600 mW, spindle-shaped cells were more stratified, and some pigment epithelium-like cells were observed. The



Figure 5. Microscopic photographs immediately after transscleral cyclophotocoagulation. (A) 600 mW. Coagulation necrosis is observed in non-pigmented and pigmented epitheliums of ciliary body. Stroma is edematous. (B) 900 mW. Coagulation necrosis is observed in ciliary epitheliums. Epithelium separates from shrunken stroma. (C) 1200 mW. Focal loss of ciliary body structure is observed. Bars = 100 mm; (A)-(C): hematoxylin-cosin staining.

collagen fibers of the stroma were loose and the number of round pigment-laden cells had decreased. At 900 mW, proliferation of spindle-shaped cells and the appearance of pigmented epithelium-like cells were observed. The hemorrhage and edema in the stroma disappeared, and cells containing pigment proliferated in a reticulate fashion. The accumulation of round pigment-laden cells still remained.

**Twenty-four weeks after surgery.** At 600 mW, one or two layers of nonpigmented epithelium-like



cells and stratified pigmented epithelium-like cells were observed, but the normal bilayer structure had not regenerated. Inflammatory change in the stroma was diminished. Proliferation of fibroblasts and regeneration of capillaries was poor in the stroma (Figure 6A). At 900 mW, regeneration of nonpigmented epithelium-like cells was not complete, and regenerated pigmented epithelium-like cells were observed only in the regions surrounding the lesions. Regeneration of capillaries was poor in the stroma, and widespread fibrosis was observed. Cells containing pig-



**Figure 6.** Microscopic photographs 24 weeks after transscleral cyclophotocoagulation. (**A**) 600 mW. One or two layers of nonpigmented epithelium-like cells and stratified pigment epithelium-like cells are observed. (**B**) 900 mW. Spindle-shaped cells are stratified. Cells containing pigment (arrow) proliferate in reticulate pattern in loose stroma. Round pigment-laden cells remain (arrowhead). Bars = 100 mm; (**A**)–(**C**): hematoxylin-eosin staining.

ment proliferated in a reticulate fashion, and some round pigment-laden cells remained (Figure 6B).

#### Discussion

Clinical observation revealed that the period over which statistically significant lowering of IOP continued was no longer than 1 week after TSCPC at 300 mW. The periods of statistically significant lowering of IOP after TSCPC at 600 mW ranged from 5 to 24 weeks. The maximum magnitude of decrease in IOP after 12 applications at 600 mW averaged 7.5  $\pm$  1.6 mm Hg; after more than 36 applications at 600 mW it ranged between 13.0  $\pm$  4.2 mm Hg and 18.5  $\pm$  14.9 mm Hg. Transscleral cyclophotocoagulation with more than 60 applications at 600 mW caused phthisis bulbi. At 900 mW, more applications resulted in longer periods of lowering of IOP. Significant lowering of IOP was obtained by TSCPC with more than 36 applications at 900 mW, but 1 of 5 eyes developed phthisis bulbi. Although TSCPC at 1200 mW was effective even with no more than 12 applications, some complications such as vitreous hemorrhage occurred. Transscleral cyclophotocoagulation at 2800 mW caused severe complications, such as cataract and rubeosis iridis, indicating that this exposure level was too high. These observations show that the most favorable parameters, causing no serious complications such as phthisis bulbi, were 600 mW and 36–48 applications, which produced mild coagulation.

Histological examination revealed that the coagulative effects of TSCPC at 300 mW in rabbits were variable, and that this exposure power was not sufficient to obtain effective coagulation spots. Transscleral cyclophotocoagulation at 600 mW caused mild coagulation, including coagulation of the epitheliums and the stroma of the ciliary body. Transscleral cyclophotocoagulation at 900 mW caused moderate coagulation, which affected the stroma more severely. With TSCPC at more than 1200 mW, the epitheliums and the stroma of the ciliary body were severely damaged.

The effects of TSCPC are due to reduced production of aqueous humor.<sup>6–8</sup> We observed regeneration of both nonpigmented and pigmented epitheliums in the early stage after TSCPC causing mild coagulation, but normal double layers could not regenerate. Stratification of regenerated epitheliums occurred but regeneration of capillaries was incomplete. These observations suggested that mild coagulation destroyed the normal epithelial-capillary complex of the ciliary body, resulting in reduction of aqueous humor and, thereby, lowered IOP.

Although the degree of lowering of IOP with moderate coagulation was almost the same as that with mild coagulation, a smaller number of applications caused phthisis bulbi. In general, ocular hypotony increases intraocular inflammatory products and damage to the blood-aqueous barrier. When this damage to the blood-aqueous barrier reaches a certain degree of severity, the production of aqueous humor begins to decrease and causes a vicious cycle.<sup>9</sup> This vicious cycle finally results in phthisis bulbi. Moderate coagulation induces more intensive and more prolonged inflammatory reactions than mild coagulation. Thus, the vicious cycle caused by inflammatory products after the lowering of IOP can more readily result in phthisis bulbi after moderate coagulation than after mild coagulation.

Some clinical studies of TSCPC using diode lasers have been reported, but treatment parameters, such as exposure power, the number of applications, and extent of exposure, have varied widely between studies. Gaasterland reported that TSCPC with high exposure power and fewer applications was less invasive because it involved less total exposure energy.<sup>10</sup> Bloom reported that TSCPC with low exposure power and greater number of applications resulted in a low incidence of phthisis bulbi.<sup>5</sup> We confirmed that TSCPC with low exposure power and greater number of applications effectively lowers IOP without severe complications such as phthisis bulbi, supporting Bloom's theory.

There are some problems with the application of our findings to clinical therapeutic use. First, the blood—aqueous barrier of rabbits is more fragile and can more easily become inflamed than that of humans.<sup>11</sup> Second, the rabbit sclera is thinner than that of humans. Third, because our experiments were performed in normal eyes, the results obtained cannot be applied to eyes with glaucoma. Although these problems must be considered, we conclude from the findings of the present study that in TSCPC of healthy pigmented rabbits, many applications at an exposure power producing mild coagulation, including coagulation of the ciliary epitheliums and only slight injury to the stroma, may provide the most effective lowering of IOP with the least possibility of serious complications such as phthisis bulbi.

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