

Gonioscopic laser sclerostomy: comparing the ablation capacities of methylene blue and reactive black stains *in vitro* and *in vivo*

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Abstract

Aim: Gonioscopic laser sclerostomy requires staining of the sclera by an iontophoresis technique to permit the absorption of the laser energy. The ablation ability of a pulsed dye laser using methylene blue as scleral stain was compared to that using reactive black in order to evaluate which stain is more effective for achieving a full-thickness sclerostomy, both *in vitro* and *in vivo*.

Materials and Methods: Iontophoresis with methylene blue and reactive black, 1% and 3% each, was performed *in vitro* on sclera from 31 autopsy human eyes. The performance of methylene blue and reactive black was studied *in vivo* both clinically and histologically. Iontophoresis of 1 eye with reactive black 3% and the fellow eye with methylene blue 3% was performed on 11 rabbits.

Results: *In vitro*, the creation of a full-thickness sclerostomy using a pulsed dye laser was significantly higher for reactive black

3% (71%) than for methylene blue 1% (40%) ($p=0.017$, McNemar's test) and methylene blue 3% (35.5%) ($p=0.0078$). *In vivo* the intraocular pressure drop (mean $\pm$ SD) was greater for reactive black 3% (-4.6 ± 6 mm Hg) than methylene blue 3% (-0.3 ± 12.9 mm Hg) ($p=0.015$, paired t-test). The frequency of bleb formation, flattening of the anterior chamber, or visible subconjunctival hole was significantly higher for reactive black (82%) than for methylene blue (18%) ($p=0.015$, McNemar's test). Histologic study in 14 rabbits immediately following GLS demonstrated 5 full-thickness sclerostomies in the reactive black-stained eyes, and 2 full-thickness sclerostomies in the methylene blue-stained eyes. The difference between reactive black and methylene blue, based on histology, was not statistically significant (Fisher's exact test).

Conclusion: Reactive black is better than methylene blue for achieving full-thickness sclerostomy *in vitro* and *in vivo*.

Introduction

Glaucoma treatment is aimed at lowering the intraocular pressure (IOP) in order to prevent damage to the optic nerve head and visual field loss. Anti-glaucoma medications, laser trabeculoplasty, and filtration surgery are the currently accepted treatments to reduce IOP.^{1,2} Recently, several laser sclerostomy techniques have been reported in the literature. The creation of a sclerostomy using an *ab externo* technique was described using a THC:YAG laser^{3,4} and using a Nd-YAG.⁵ This technique requires the insertion of a probe into the subconjunctival space. Several *ab interno* techniques using the argon,⁶⁻¹¹ erbium YAG,^{12,13} excimer,¹⁴⁻¹⁵ and Neodymium-YAG lasers¹⁶⁻²⁰ have also been

described. These techniques require the passage of a fiber-optic or sapphire probe across the anterior chamber.

Ab interno laser sclerostomy with the laser energy delivered through a gonioscope was first described by March et al^{21,22} and modified by Latina et al.^{23,24} A sclerostomy is created for drainage of aqueous from the anterior chamber into the subconjunctival space in a noninvasive approach. The modified technique utilizes staining of the sclera by iontophoresis to enhance laser energy absorption by the sclera. Methylene blue (MB) has been used by Latina et al^{23,24} as a scleral stain, but scleral ablation was inconsistent. The use of a different stain may improve the ablation capacity of the laser and the achievement of

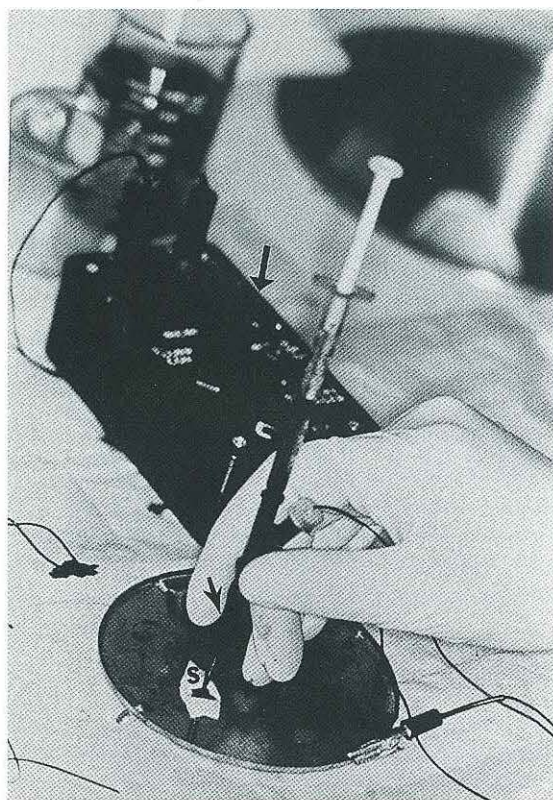


Figure 1. Iontophoresis of scleral strip. (S) sclera strip and (Arrow) iontophoresis apparatus.

full-thickness sclerostomy (FTS).^{25,26} Reactive black (RB) was suggested since it can be iontophoresed effectively and its maximal absorption is in the visible light range.²⁷ This study was conducted to compare the ablation capacity of MB and RB both *in vivo* and *in vitro* in order to evaluate which stain is more effective for creating full-thickness gonioscopic laser sclerostomy (GLS).

Materials and methods

A flashlamp-pumped pulsed-dye laser was provided by Candela Laser Corp, Wayland, MA. The laser output was coupled to a slit lamp delivery system by a fiberoptic cable that provided a spot size of 200 μm . An 8 μsec pulse duration at a wavelength of 590 nm and an energy of 250 ± 5.5 mJ were used. Prior to treatment, the laser output was measured by an energy meter (Model 365 power and energy meter, Scientech, Boulder, CO). The laser was aimed via a helium neon beam focused on the center of the stain spot, and triggered with a foot switch in a single-pulse mode.

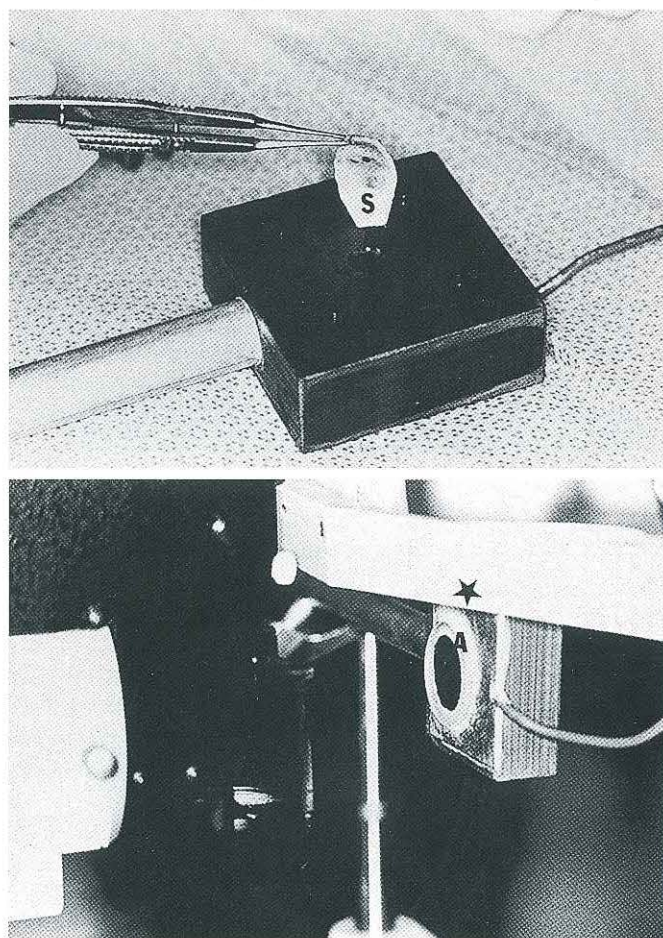
Iontophoresis was performed on the sclera using a constant current source and an iontophoretic probe (Candela Laser Corp, Wayland, MA, Figure 1). The probe used in our study consisted of a micropipette tip with a surface area of 0.13 mm², a plastic Y-shaped connector which contained an Ag-AgCl electrode within a sidearm lumen and a 1-cc syringe as a reservoir for the stain. The electrode was connected by a conducting wire to the power supply. For RB, which is negatively charged, the positive electrode of the current source served as a ground, and the negative electrode was placed on the iontophoresis probe. For MB, which is positively charged, the electrode connections were reversed. A current of 500 mA and a duration of 5 minutes were used for the iontophoresis of each stain. The maximal absorption of both MB and RB in the concentration used was within the range of the wavelength of the delivered laser energy (590 nm).^{25,26}

Two studies

Thirty-one frozen autopsy human eyes provided by Arizona Lions Eye Bank were thawed at room temperature. The cornea, lens, and uvea were removed using Wescott scissors and a scalpel. The sclera from each eye was sectioned into six equal strips, each 0.5 x 1.5 mm. The scleral strips were soaked in 0.9% sterile saline solution (Baxter Healthcare Corp, Deerfield, IL) for 10 minutes.

Each of the six scleral sections was randomly selected to be iontophoresed by MB 1% and 3% (American Reagent Laboratories, Shirley, NY), salted reactive black (SRB) 1% and 3% (Aldrich Chemical Co, Inc, Milwaukee, WI), and pure reactive black (PRB) 1% and 3% (purified from SRB by Candela Laser Corp, Wayland, MA). Iontophoresis was performed using each of the stains with the above-mentioned parameters. A 'mock anterior chamber' was constructed of plexiglass (5 x 5 x 1.9 cm), with a glass window on one side and an aperture on the opposite side. The scleral strip was placed at the aperture (Figures 2A and 2B). The normal anatomic orientation of the sclera was maintained, with the episcleral surface facing out. The chamber was filled with balanced salt solution (Alcon Surgical, Fort Worth, TX) to simulate the aqueous humor. The laser beam was focused on the stained inner surface of the sclera and laser energy was delivered until a full-thickness sclerostomy was achieved or to a maximum of 80 shots.

The creation of a full-thickness sclerostomy was confirmed by direct visualization through the slit lamp, retroillumination, or flow of saline through the hole. Success in achieving full-thickness sclerostomy with different stains was evaluated.



Figures 2A and 2B. "Mock anterior chamber." (A) aperture, (S) scleral strip, and (*) holder.

In scleral strips from 23 autopsy eyes, re-iontophoresis at the same site was performed in the samples that failed to achieve full-thickness sclerostomy and the laser treatment was repeated using the same parameters. Success in achieving full-thickness sclerostomy following re-iontophoresis was evaluated.

Two randomly selected scleral strips from each stain group were fixed with 10% neutral-buffered formalin for 48 hours, embedded in paraffin, serially sectioned at 5 μ thickness, stained with hematoxylin-eosin, and examined histologically.

McNemar's test, taking into account the association of measurements between eyes, was used to compare the ablation rates for the different stains and stain concentrations.

In vivo studies

Gonioscopic laser sclerostomy (GLS) was performed in a rabbit model. This study was performed with the approval of the UCLA Animal Research Committee on the basis of the *in vitro* experiment results, the *in vivo* performance of GLS using MB 3% was compared, both clinically and histologically, to that using SRB 3%.

In order to establish the clinical changes following GLS, 11 pigmented rabbits weighing between 1.5 and 3.0 kg were studied. Preoperatively, IOP was measured in both eyes by pneumotonometry (Alcon Applanation Pneumotonograph, Digilab Inc., Cambridge, MA) under topical 0.5% proparacaine anesthesia (Alcon, Humacao, Puerto Rico), taking the mean of three separate readings. External examination of conjunctival injection, corneal clarity, anterior chamber reaction, and lens clarity was performed with a Zeiss slit lamp (Carl Zeiss, Germany). General anesthesia was then given using ketamine 50 mg/kg IM (Parke Davis, Morris Plains, NJ), and xylazine 15 mg/kg IM (Moby Corp, Shawnee, KS).

One eye of each rabbit was randomly selected to undergo iontophoresis with MB 3%, and the fellow eye underwent iontophoresis with SRB 3%. Topical proparacaine 0.5% was applied for 5 minutes via a cotton tip applicator to the planned site of iontophoresis to enhance stain penetration.²⁷ Iontophoresis of the sclera was performed superiorly at the limbus. The IOP was measured again immediately before the laser treatment.

The stained spot was viewed through a gonioscopes (Haag-Streit AG, Berne, Switzerland). The stained area was always at the trabecular meshwork and the insertion of the iris pillars to the cornea. Laser energy was delivered repeatedly to the center of the stained area, with a maximum of 80 shots.

Immediately following the laser procedure, IOP was measured. Bleb formation, anterior chamber depth, and creation of a visible subconjunctival scleral hole were observed using a surgical microscope at 25 X magnification (Carl Zeiss, Germany).

In eyes that failed to show a drop in IOP of at least 5 mm Hg, bleb formation, shallowing of the anterior chamber, or scleral hole, re-iontophoresis was performed at the same site and laser treatment was repeated using the same parameters.

Other signs documented were the darkness of the stained spot (good = intense dark stain spot; bad = light or absent stain spot), bleaching of the stained spot during the laser procedure, bubble formation in the anterior chamber, hyphema, and subconjunctival hemorrhage. All of the animals were sacrificed with an overdose of sodium pentobarbital 75 mg/kg IV immediately after the laser treatment for postoperative examination.

In order to examine the histological effect of GLS, iontophoresis was performed on both eyes of seven rabbits using MB 3% and both eyes of seven rabbits using SRB 3%. The procedure performed was the same as that described above, except that re-iontophoresis was not done. Immediately following the laser treatment, the animals were sacrificed. The eyes were enucleated and fixed in 10% neutral buffered formalin for 48 hours. The eyes were then sectioned in the sagittal plane into two calottes, and embedded in paraffin. Serial sections 5 microns thick were obtained and stained with hematoxylin-eosin. The sections were studied for the existence of a sclerostomy under a microscope with 40 X magnification (Carl Zeiss, Germany).

McNemar's test was used to analyze the difference between MB 3% and SRB 3% with respect to the positive clinical parameters of bleb formation, shallowing of the anterior chamber, and visible scleral hole. A paired t-test was used to analyze the difference between MB 3% and SRB 3% in the reduction of IOP immediately after the procedure. Fisher's exact test was used to analyze the difference between MB and SRB groups in achieving histologically proven full-thickness sclerostomy.

Results

In vitro studies

The frequency of achieving a full-thickness sclerostomy in autopsy human sclera was highest using SRB 3% and lowest using MB 3%. (Figure 3). Higher concentrations of SRB and PRB had higher rates of full-thickness sclerostomy, but SRB and PRB were not significantly different statistically. The salt ions did not appear to make a difference in iontophoresis. The differences between SRB 3% and either MB 1% or MB 3% in achieving full-thickness sclerostomy were statistically significant using McNemar's test of difference ($p=0.017$ and 0.0078 , respectively).

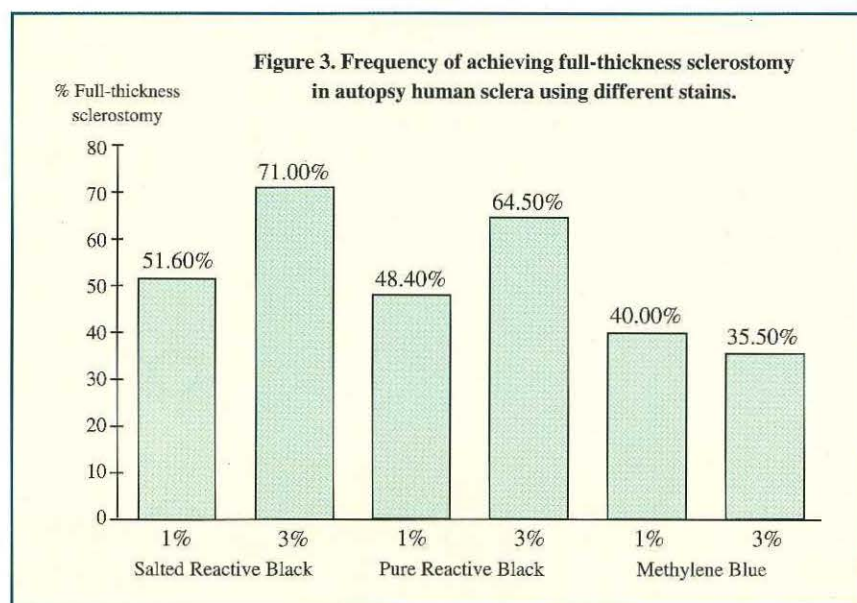


Table 1: Re-ontophoresis and re-laser performed on failed samples of autopsy human sclera

	Number of samples that underwent re-ontophoresis	Successful sclerostomy following re-ontophoresis	
		Number of samples	% of samples
SRB 1%	11	8	36.40
SRB 3%	8	5	62.50
SRB 1%	10	2	20.00
SRB 3%	7	3	42.90
SRB 1%	14	6	42.90
SRB 3%	13	2	15.40

SRB = Salted reactive black; PRB = Pure reactive black; MB = Methylene blue

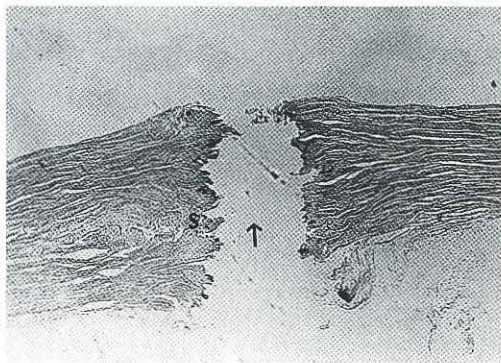


Figure 4. Histological sample of a full-thickness sclerostomy in autopsy human sclera. (S) sclera and (Arrow) sclerostomy. (X 40)

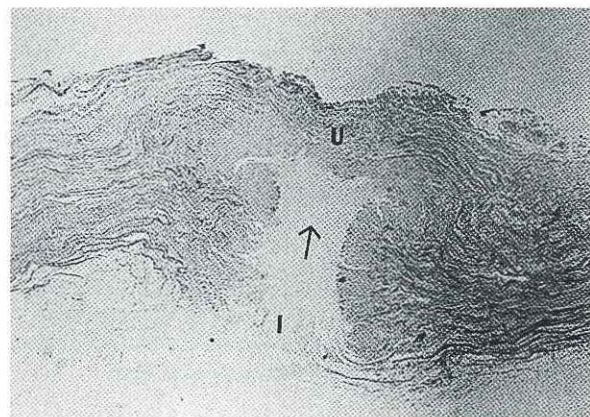


Figure 5. Histological sample of a partial-thickness sclerostomy in autopsy human sclera. (Arrow) sclerostomy, (I) internal side of sclera, and (U) outer side of sclera. (40 X)

Re-ontophoresis and laser retreatment performed on scleral samples from the 23 autopsy eyes were successful in 62.5% of scleral strips stained with SRB 3% and 15.4% of scleral strips stained with MB 3% (Table 1). The success rate for achieving full-thickness sclerostomy following both iontophoresis and re-ontophoresis was the highest using SRB 3% (87%) and the lowest using MB 3% (52.2%) ($p=0.0084$ McNemar's test (Figures 4 and 5). Bleaching during the procedure was observed in 69.2% of scleras stained with MB 3% and 18.2% of scleras stained with SRB 3%. Therefore, SRB 3% was selected for the in vivo studies.

In vivo studies

The decrease in IOP immediately following laser treatment in the rabbit model was significantly greater in eyes iontophoresed with SRB 3% (mean $\pm$ SD, 4.6 ± 6.0 mm Hg) than in those iontophoresed with MB 3% (0.3 ± 12.9) ($p=0.015$, paired t-test) (Figure 6). Following the laser procedure, the frequency of flattening of the anterior chamber, bleb formation, or scleral hole visualization was greater

Figure 6. Change in intraocular pressure immediately following gonioscopic laser sclerostomy in the rabbit model: reactive black 3% vs. methylene blue 3%.

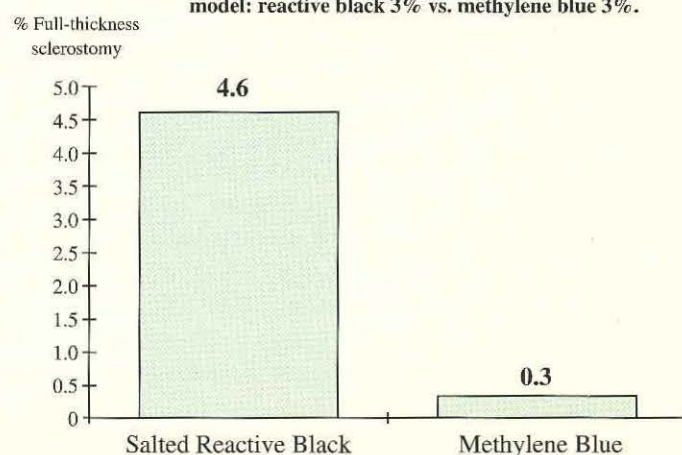


Table 2: Clinical parameters following GLS: salted rReactive black 3% vs. methylene blue 3% in the rabbit model.

	SRB 3% (%)	Number of eyes	MB 3% (%)	Number of eyes	p Value
Darkness of stain spot (good)	100.0	11	81.8	9	0.75
Anterior chamber bubbles	90.9	10	63.6	7	0.24
Bleb	81.8	9	0.0	0	0.0027*
Hyphema	54.5	6	36.4	4	0.32
Subconjunctival hemorrhage	54.5	6	36.4	4	0.71
Flattening anterior chamber	45.5	5	18.2	2	0.26
IOP decrease >5 mm Hg	45.5	5	36.4	4	0.66
Visible scleral hole	45.5	5	0.0	0	0.025*
Bleaching of the stain	45.5	5	72.5	8	0.25
IOP increase >5 mm Hg	0	0	45.5	5	0.26*

* Statistically significant ($p < 0.05$)

Table 3. Histological findings following GLS in the rabbit model.

	Full-thickness sclerostomy Number of eyes	Partial-thickness sclerostomy Number of eyes	No effect Number of eyes	Total
SRB 3%	5	9	0	14
MB 3%	2	10	2	14

SRB = salted reactive black; MB = methylene blue

for eyes stained with SRB 3% than for eyes stained with MB 3%. (Table 2). The frequency of at least one positive clinical success criterion was significantly higher for SRB 3%-stained eyes (81.8%) than for eyes stained with MB 3% (36.4%) ($p = 0.025$, McNemar's test). Re-ontophoresis and laser retreatment in the rabbit model did not change any of the clinical success criteria. The differences between MB 3% and SRB 3% with respect to the frequency of bubble formation in the anterior chamber, hyphema (less than 1/3 anterior chamber volume), subconjunctival hemorrhage, darkness of the stain spot, and bleaching of the stain were not statistically significant (Table 2).

Full-thickness sclerostomy following GLS was histologically demonstrated in 5 out of 14 eyes iontophoresed with SRB 3% and in 2 out of 14 eyes iontophoresed with MB 3% (Table 3, Figure 7). The differences between SRB 3% and MB 3% with respect to histological findings of full-thickness, partial-thickness, or no sclerostomy were not shown to be statistically significant by Fisher's exact test.

Discussion

Gonioscopic laser sclerostomy uses staining of the sclera by iontophoresis to enhance absorption of laser energy for creation of a full-thickness sclerostomy. Optimal staining of the sclera is essential for ablation of the tissue by the laser. The stain used should be

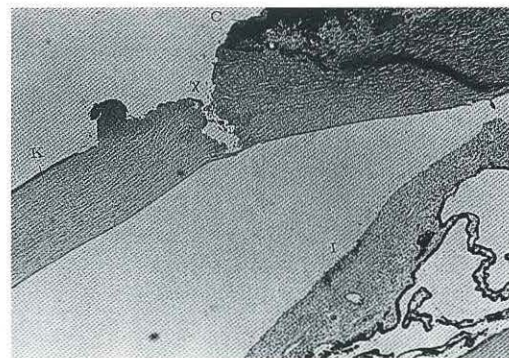


Figure 7. Full-thickness sclerostomy in the rabbit model using RB 3% as a scleral stain. (C) conjunctiva, (X) sclerostomy, (K) cornea and (I) iris. (25 X magnification)

iontophoresed completely throughout the scleral target area and have an absorption peak within the range of the laser energy. MB and SRB are good stains for iontophoresis since they are both charged at the physiological eye pH, have relatively low molecular weight (MB=542 d, SRB=991.8 d), and are water soluble and lipid insoluble.²⁵⁻²⁶

Gonioscopic laser sclerostomy has several potential advantages over trabeculectomy and other laser techniques for the creation of

sclerostomies. The *ab externo* technique with a THC:YAG laser is associated with trauma to the conjunctiva secondary to the insertion of a probe.³⁻⁵ The *ab interno* techniques⁶⁻¹⁹ are associated with a risk of damage to intraocular structures when the probe crosses the anterior chamber. For instance, Jaffe et al⁶ found corneal edema and focal iris atrophy. Higginbotham et al¹² found corneal damage, hyphema, iris burns, focal cataracts, corneal pannus, and ruptured blebs in a significant number of cases. In GLS, since laser energy is delivered through a gonioscope, the approach is non-invasive. Hence, fewer intraoperative and postoperative complications such as flat anterior chambers, or infection are expected. The risk that bleb scarring will follow the procedure may be reduced by this less invasive approach.

Latina et al^{23,24} using GLS and MB 1% as a scleral stain, studied the number of pulses required to perforate excised human sclera. Their *in vitro* study demonstrated that the ablation efficiency increased with shorter pulse durations. Using a 1.5 μ sec pulse duration, 100 μ m spot diameter, and 75-100 mJ pulse energy, 85%(5/6) sclerostomies were achieved. Using parameters that were the same, aside from a 20 μ sec pulse duration, no perforation (n=4) was achieved. They also demonstrated that using higher energy increased the success rate. For example, using a 20 μ sec pulse duration, a 200 μ sec spot diameter, and a pulse energy of 100 mJ, no perforation was achieved in 4 rabbit eyes. However, using the same parameters with a laser energy of 250-275 mJ, sclerostomy was achieved in 4 out of 5 rabbits' eyes (80%). Since Latina et al used freshly enucleated human eyes, a different 'mock anterior chamber,' different iontophoresis parameters (400 μ A), and a different laser wavelength (660 nm), their results may not be directly comparable to the results presented in this study.

March,²¹ using a Q-switched Neodymium-YAG laser, produced a corneoscleral perforation in 6 human cadaver eyes. An "optimal" perforation in 100% of the cases required 26,676 mJ total applied energy (our maximum was 20,000 mJ). An 8-mm corneal trephine section was performed so that the trabecular meshwork could be exposed in an empty anterior chamber. Removal of the cornea may have decreased the energy required to create a full-thickness sclerostomy.

Hoskins et al⁴ used a THC:YAG laser to create a thermal sclerostomy in 21 glaucomatous eyes of 19 patients. A 1 mm conjunctival stab incision was required to insert the probe. Pulse energies of 80 to 120 mJ were used. The total energy required to create a full-thickness sclerostomy was less than 5000 mJ. Of the 21 treated eyes, 12 (57%) were successful 3 months after the first procedure. Although the energy used in this procedure was relatively low, important complications such as choroidal effusion and iris prolapse into the sclerostomy site were observed.

Early clinical studies of GLS in glaucoma patients have demonstrated limited success with MB stain.²⁸⁻³⁰ Therefore, other stains have been considered and compared to MB for iontophoresis and laser ablation.^{31,32} RB stain has been found to be an attractive alternative because of its physiochemical properties.³² Initially, there was concern that the salt ions in RB stain may limit the concentration of stain delivery into sclera by iontophoresis, but this was shown to have no significant effect in this study. MB stain's laser ablation efficiency is limited by photobleaching, and this is much less for RB stain. The threshold for

laser ablation of MB is 0.0625%³¹ compared to 0.001% for RB.³² Therefore, RB absorbs laser energy at 590 nm better and ablates sclera more efficiently than MB stain *in vitro*.

The 'mock anterior chamber' is a valuable *in vitro* model for studying and better understanding the interaction between the laser energy and the stained human sclera under controlled conditions. However, as a model, the autopsy human sclera mounted in a 'mock anterior chamber' has several limitations. The lack of debris from adjacent ocular structures during the laser treatment, the direct visualization of the stained sclera instead of indirect visualization provided by the gonioscope, and the delivery of laser energy perpendicular to the surface of the tissue instead of at an oblique angle as in the eye, were optimal conditions created *in vitro* that are not present *in vivo*. This may explain why the *in vitro* model showed greater success in achieving full-thickness sclerostomies than the *in vivo* model.

Re-iontophoresis increased the success rate of achieving full-thickness sclerostomy in autopsy human sclera. This may be explained by the fact that the main reason for failure in the SRB-stained scleras was ineffective iontophoresis. Since full-thickness sclerostomy was not achieved in all of the scleral strips following re-iontophoresis, there may be additional reasons for failure. One possible reason is a change in the stain during the laser procedure, such as bleaching of the stain in the course of laser treatment. This bleaching was observed less frequently in our study when SRB was used than when MB was used. Another possible factor is a difference in the interaction between the stain and laser energy in dead tissue and in living tissue or staining variability of the sclera.

In vivo, lowering of intraocular pressure, flattening of the anterior chamber, and bleb formation immediately following the laser procedure were more likely to occur with the use of SRB 3% stain than with the use of MB 3%. These clinical findings were supported by the histological findings. Elevations of IOP greater than 5 mm Hg were more frequent in the MB 3% eyes than in the SRB 3% eyes. This disparity may be explained by the histological findings, which showed a lower frequency of full-thickness sclerostomy and higher frequency of no sclerostomy in the MB 3% stained eyes.

There are no obvious reasons for not achieving full-thickness sclerostomy in all cases treated by pulsed dye laser. The iontophoresis may not deliver a consistently sufficient stain concentration, even though a visually uniform dark dye spot was present. This hypothesis was suggested by the histological findings of unperforated tissue at the internal aspect of the sclera, which is the last location to be stained. The debris circulating in the anterior chamber around the treated area and adjacent structures during the laser treatment may decrease the amount of energy delivered to the treated site. This hypothesis is suggested by the failure of re-iontophoresis and re-laser to increase the clinical success criteria *in vivo*.

The purpose of this study was to determine which of the tested stains is more effective in ablating the sclera with a pulsed dye laser. Our results show that scleral ablation is more successful both *in vitro* and *in vivo* following iontophoresis with SRB. Since staining of the sclera is essential for absorption of the laser energy, using SRB may improve the clinical effectiveness of GLS. ●

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