Histologic Effects of Contact Ultrasound for the Treatment of Glaucoma

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The histologic effects of a contact ultrasound applicator were compared with those of the immersion applicator that is currently used clinically for the treatment of glaucoma. The applicator coupling cone uses a distensible rubber membrane that can be inflated to control stand-off distance relative to the surface of the eye. This feature allows the focal point of the therapeutic beam to be placed at selective depths. Histologic comparisons of lesions in rabbit and pig eyes showed lesions in the sclera and ciliary body that were similar to those produced by the immersion transducer when the same focal position was used. Moving the focal point to a greater depth resulted in less superficial damage, yet still produced ciliodestruction. Damage to the blood supply of the ciliary body, as found in human cadaver eyes, may be an additional mechanism of action of therapeutic ultrasound, and perhaps of other transscleral high-energy modalities. Invest Ophthalmol Vis Sci 32:2136-2142, 1991

The efficacy and safety of high-intensity focused ultrasound in the treatment of glaucoma have been well established, with reported success rates between 50% and 65% after a single treatment. Retreatment can be used if intraocular pressure is uncontrolled, thus increasing the effective success rates.1-7

The current treatment technique involves the use of a waterbath. The use of a contact method would simplify patient preparation and treatment.

We compared the results of treatment with a contact ultrasound device in animal and cadaver models with those produced by the immersion-bath therapeutic ultrasound system. In addition, we examined histologic effects to elucidate the mechanisms by which ultrasound and other transscleral high-energy modalities exert their pressure-lowering effect.

Our previous work with a rabbit model established 8

Materials and Methods

Twelve 1.5–2.5 kg male and female adult New Zealand white rabbits were divided into three groups to receive various types of treatment.

Group 1 received two applications in one eye, with focal point depths of 0, 1, and 2 mm from the surface of the eye. These studies were used to compare lesions produced by different treatment depths.

Group 2 received two applications in one eye, with a focal depth of 1 mm, at lateral distances of 0, 1, and 3 mm from the limbus. These studies were conducted to determine the optimal treatment position for producing ciliary body lesions in the rabbit.

Group 3 received two applications in one eye, at the limbus, with treatment durations of 1, 2, and 3 sec.

Two 10.0-kg pigs (Minipig Yucatan, Charles River Laboratories, Inc., Wilmington, MA) received one contact treatment and one immersion treatment in each eye; one eye was treated 2 weeks before death and the other was treated immediately before death.
Both animals received all treatments 2–3 mm from the limbus. Thus, a comparison could be made between the two techniques in terms of long-term effects.

Human cadaver eyes (<48 hr postmortem, no prior intraocular surgery) were treated with the contact transducer at distances of 1.5 and 2 mm from the limbus, with a focal point depth of 2 mm.

These procedures adhered to the ARVO Resolution on the Use of Animals in Research.

Transducers

The contact transducer is a ceramic (PZT-4, Channel Industries, Santa Barbara, CA), cylindrical shell with a resonant frequency of 1.4 MHz, an outer diameter of 50 mm, and a radius of curvature (distance to focus from inner surface) of 56 mm. A schematic diagram of the device is shown in Figure 1. We operated the transducer at its third harmonic, or at 4.6 MHz. The therapeutic transducer is housed within a cylindrical cone that is filled with degassed distilled water and is contained by a distensible rubber membrane (Schmid Laboratories, Little Falls, NJ). A valve and a water-filled syringe are attached to the cone. Injection or withdrawal of water alters the position of the membrane, thereby controlling stand-off distance to the surface of the eye. A set of three positioning pins is used to contact the globe. These pins can be adjusted, and their tips have a set location with respect to the focal point of the therapeutic transducer. The membrane is coupled directly to the eye with Goniosol [Hydroxypropylmethylcellulose 2.5%] (IOLAB Pharmaceuticals, Claremont, CA). The immersion-bath transducer was described previously.9

![Fig. 1. Schematic diagram of contact therapeutic ultrasound assembly.](image)

Table 1. Treatment parameters

<table>
<thead>
<tr>
<th>Model</th>
<th>Frequency (MHz)</th>
<th>Force (grams)</th>
<th>Method</th>
<th>Focal Depth (mm)</th>
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<td>2.0</td>
<td>Contact</td>
<td>0, 1, and 2</td>
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<td>3.2</td>
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<td>0</td>
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![Fig. 2. (A) Ultrasound-treated rabbit eye. Contact method; 1 mm posterior to the limbus; immediately post-treatment; section through lesion center. Epithelium of conjunctiva is focally denuded (small arrow), and its stroma is coagulated (large arrow). Sclera (S) is coagulated and swollen. Ciliary body stroma of pars plana area and part of choroid are also coagulated. Ciliary epithelium at pars plana region and peripheral retina are partially damaged (curved arrow). Hematoxylin and eosin, ×41. (B) Ultrasound-treated pig eye (immersion method; 2 mm posterior to limbus; immediately post-treatment; section through lesion center). Conjunctival epithelium and stroma are focally defective (arrow) at the beam path, but remaining conjunctival stroma is not coagulated. Sclera (S) is coagulated and swollen. Epithelium and stroma of pars plana area show coagulative damage. Hematoxylin and eosin, ×41.](image)
Ultrasound Treatment

The rabbits were subcutaneously sedated with a 1:1 mixture of Rompun (20 mg/ml Xylazine Mobay Corp., Shawnee, KA) and Ketalar (100 mg/ml Ketamine, Parke-Davis, Morris Plains, NJ). A 0.5% Proparacaine, Alcon, Humacao, PR drops were used for corneal anesthesia. The pigs were premedicated via intramuscular (IM) injections 25 min before general anesthesia was induced with atropine 0.04 mg/kg and Azaparone 2 mg/kg (Pitman-Moore, Mundelein, IL). Ketamine 10–15 mg/kg IM was administered 10 min later. Inhalational anesthesia was obtained using a vaporizer filled with 2% Isoflurane (Anaquest, Madison, WI). After the procedure, the pigs were given oxygen before they were returned to the cage.

Each rabbit was placed on its side. The eye to be treated was proptosed as described by Ortiz and associates. In the pig, the lids were retracted with a Barraquer speculum (Storz Instrument Co., St. Louis, MO), and the eye was rotated into the desired position with a cotton swab after the area was prepared as previously described. After injection of 0.9% saline to obtain normal tension, the cadaver eye was placed in a holder and treated as described above.

As the cone was placed on the globe mild pressure was exerted until the three offset pins were in contact with the surface of the eye. This technique ensured a perpendicular orientation that was relative to the surface of the eye and placement of the focal point at a known depth. To obtain different depths, the pin positions were adjusted and calibrated before exposure. Treatment parameters are shown in Table 1.

The treated animal eyes were given Decadron (dexamethasone sodium phosphate, Merck, Sharp and Dohme, St. Louis, MO) ointment immediately after insonification. Demerol 2 mg/kg IM was given to the pigs if postoperative pain was evident.

Specimen Preparation

After treatment, the animals were killed with a barbiturate-based euthanasia solution. The eyes were then enucleated with the bulbar conjunctiva intact. Rabbit and human cadaver eyes were fixed in 10% formalin. Pig eyes were first fixed in 3.5% glutaraldehyde in 0.5 molar phosphate buffer and then transferred to 10% formalin. After fixation, the eyes were opened. Each ultrasound-treated lesion, together with surrounding tissue, was excised. These tissues were embedded in paraffin and serially cut, in the merid-

Fig. 3, Ultrasound-treated pig eyes. (A) (Immersion method; 2 mm posterior to limbus; 2 weeks post-treatment; section through lesion center). (B) Contact method; 2 mm posterior to limbus; 2 weeks post-treatment; section through lesion center. In (A) and (B), conjunctival epithelium is recovered and in the stroma, mild fibroblast-proliferation and mononuclear cells are found. Slight thinning of the sclera (between arrows) is noted in both. In deeper portions, many pigment-laden cells are found in both. Although a focal separation of ciliary epithelium (pars plana) (A) and focal detachment of atrophic peripheral retina (B) are noticed in innermost layers, these differences are due to slight differences in beam centers at time of treatment. * indicates artifactitious separations. A and B; Hematoxylin and eosin, X95.
Fig. 4. (A) Ultrasound-treated rabbit eye. Contact method; 3 mm posterior to limbus; focal depth 1 mm; immediately after treatment; section through lesion center. Conjunctival epithelium is denuded and its stroma (arrow) is coagulated and swollen with some large vacuoles. Sclera (S) is coagulated with swelling; underlying choroid and peripheral retina also are coagulated. Hematoxylin and eosin, X41. (B) Ultrasound-treated rabbit eye. Contact method; at limbus; focal depth 2 mm; immediately post-treatment, section through lesion center. Conjunctival damage is minimal with no stromal coagulation (arrow), whereas sclera is mildly coagulated and swollen. Severe coagulative damages are found in both the epithelium and stroma of ciliary processes (curved arrow); focal coagulative changes are found in pars plana and iris. Hematoxylin and eosin, X41.

Results

Clinical Changes

Rabbit: The treatments with the superficial focal zone position usually produced a 1–3-mm area of blanching, with a central spot of translucency in the sclera. There was marked chemosis of the conjunctiva that peaked at 24 hr. Treatment with the deeper focal zone position, however, caused a slightly blanched, nontranslucent spot at the center of the beam. The degree of chemosis at 24 hr was significantly reduced.

Pig: Similar lesions were produced with both the contact and immersion techniques, but without a marked degree of chemosis. Occasionally, a small subconjunctival hemorrhage resulted with both techniques.

Cadaver: There was little effect on the conjunctiva, except for some mild blanching and retraction.

Histopathologic Features

A comparison of contact and immersion techniques showed that the lesions produced were similar, with the exception of more pronounced coagulative changes in the conjunctival stroma with the contact transducer (Fig. 2). Within 2 weeks, however, the conjunctival epithelium healed, and the stromal changes became essentially the same in both groups (Fig. 3). For all treatment depths, coagulative damage and swelling of the conjunctiva, sclera, and ciliary body were consistent findings. At a more superficial treatment position (0–1 mm focal depth), thermal damage to the conjunctiva tended to be more pronounced (Fig. 4A). The deepest focal position (2 mm) produced minimal damage to the conjunctival tissues. When the probe was positioned at the limbus of the rabbit eye, this caused the most severe damage to the ciliary processes (Fig. 4B).

In the rabbit eye, only treatment directly through
the limbus resulted in damage to the pars plicata region of the ciliary body (Fig. 4B). Treatments at 1 mm and 3 mm from the limbus produced lesions in the pars plana (Fig. 2A) and peripheral retina (Fig. 4A), respectively.

In the cadaver eyes, treatments that were 2 mm posterior to the limbus resulted in damage to the pars plana and pars plicata regions of the ciliary body (Fig. 5). In the latter regions, coagulation of the collagenous tissue surrounding the blood vessels in the stroma was seen (Figs. 5C,D). Treatment at 1.5 mm posterior to the limbus produced similar coagulative stromal change that was more confined to the pars plicata region. With this position, however, a focal peripheral cataract (due to coagulation) was consistently seen near the treatment site; on the other hand, in the former position (2 mm from the limbus), no cataract was produced in three of four lesions studied.
although a small coagulative spot in the lens periphery was found in one lesion.

Discussion

Our studies showed that lesions produced by the contact transducer assembly are histologically similar to those produced by the immersion transducer. Also, the location of the lesions relative to the surface of the eye is directly related to the depth of the focal point of the ultrasound beam, and an increased focal-point depth produced lesions in the ciliary body while minimizing superficial damage. As shown previously with the immersion transducer, the size and extent of the lesions are related to the duration of treatment.

The distribution of thermal damage among the pars plicata, pars plana, and peripheral retina is related to the distance of the treatment site from the limbus. Additionally, there are interspecies differences. In the human cadaver eye, treatment at 1.5–2 mm from the limbus affects the pars plicata and the pars plana; 2 mm posterior to the limbus appears to be the optimal treatment distance because this process prevents damage to the lens. A similar spatial relation was reported in eyes obtained at autopsy and treated with YAG laser (neodymium:YAG).12

Despite the ability of ultrasound treatment to reduce intraocular pressure, the mechanism of action is not clearly understood. Previous theories, including those assumed by us on the basis of our experiments are listed: 1) destruction of the ciliary epithelium, resulting in decreased aqueous production, 2) enhanced uveoscleral outflow, 3) formation of a new outflow tract (from the posterior chamber through necrotic pars plana and thinned sclera), and 4) widening of the interspaces of the trabecular meshwork by pulling of the contracted scar tissue in the pars plana. Among these, destruction of the ciliary epithelium would seem to be significant, as actually seen in human eyes.14,15

Some evidence, however, seems to dispute direct ciliary epithelial damage as the primary mechanism in either therapeutic ultrasound or photoocoagulation when performed at 2–3 mm posterior to the limbus, the commonly used therapeutic distance. Although the energy-delivering mechanism and extent of damage differ between therapeutic ultrasound and photoocoagulation, both techniques may have similar tissue-destructive properties. Schubert pointed out that noncontact transscleral YAG treatments at 2 mm posterior to the limbus, presumably overlying the ciliary body, require more treatment than those at 3 mm.16 We also have seen infrequent damage to the ciliary epithelium of pars plicata regions in pig eyes that were treated at 2 mm posterior to the limbus.11 This finding, however, may be attributable to a species difference because we saw damage to the pars plicata in human cadaver eyes that were treated at the same distance in this study. Schubert and Federman17 proposed an inflammation theory to explain the pressure reduction after treatment with either photoocoagulation or cryopexy. Our observation of severe perivascular coagulative change in the ciliary body stroma at the region of the pars plicata of the cadaver eye is similar to changes seen in experimental and human eyes after cryotherapy.18 Although our observation was limited to the nonperfused human eye, these changes could cause inadequate blood supply for the ciliary processes and result in a functional crippling of the ciliary epithelium, even without direct damage to the epithelium and, thus, decrease production of aqueous humor.

The device described in this report combines the benefits of ultrasound with the convenience of other handheld techniques. The contact transducer system is easier and faster, yet it produces histologic effects similar to those produced by the immersion system. Clinical studies with this device are forthcoming.

Key words: glaucoma, therapeutic ultrasound, histopathologic structures, cyclodestructive surgery, contact method

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References


