In Vitro Contractility of Avascular Corneal Wounds in Rabbit Eyes

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Contraction of corneal wounds has been the topic of recent speculation, particularly in reference to regression of corneal flattening following radial keratotomy. In an animal model of corneal wound fibroplasia, we offer the first demonstration of in vitro contractility by avascular corneal wound tissue. Three millimeter diameter full-thickness corneal trephine wounds were made in 17 New Zealand white rabbit eyes. The animals were killed and specimens were extracted 3–4 wk postinjury. Contractile responses of corneal wounds were measured on a microdynanage force transducer. When exposed to serotonin, epinephrine or norepinephrine corneal wounds showed contractions reaching maximum force of 20–100 mg with a peak response obtained within 5–10 min and persisting several hours. Normal corneas did not respond to any agent. All normal iris muscle specimens contracted to acetylcholine exhibiting peak responses of 30–60 mg of force within 5 sec decaying over the following 10–20 min. This is different from corneal wounds which fail to respond to acetylcholine (P < 0.005). These data suggest that avascular corneal wounds possess contractile properties. Invest Ophthalmol Vis Sci 26:1449–1452, 1985

The recent development of keratorefractive surgery, particularly radial keratotomy, has brought increased importance to the study of corneal wound healing. Regression of the corneal flattening effects of radial keratotomy is a particularly interesting finding which is most likely related to the corneal wound healing process. Contraction of corneal wounds has been implicated as a possible etiologic factor involved in this loss of effectiveness. Wound contraction has often been described during the healing process, particularly in association with neovascularized granulation tissue and inflammatory membranes. However, to date there has been no published report demonstrating contractile properties of avascular corneal wounds. Herein we present the first report of contractility in avascular corneal wound tissue, demonstrated in vitro in an animal model.

**Materials and Methods.** New Zealand White rabbits weighing 3–4 kg were used in this study. All care and handling of these rabbits conformed to the ARVO Resolution on the Use of Animals in Research. Following sedation with intravenous sodium pentobarbital, a 3-mm diameter full-thickness trephination wound was made in the central cornea of one eye in each of 17 rabbits. Neosporin ophthalmic ointment was used to prevent contamination of the corneal wound.

**Table 1.** Contractile responses to pharmacologic agents

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<tr>
<th>Tissue/agent</th>
<th>P*</th>
<th>5-HT</th>
<th>NE</th>
<th>E</th>
<th>ACh</th>
<th>V</th>
<th>H</th>
<th>BSS</th>
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<tbody>
<tr>
<td>Cornea</td>
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<td></td>
<td></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Normal</td>
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<td>0</td>
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<tr>
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<td>(0/6)†</td>
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<td>(0/6)</td>
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<td>(0/6)</td>
<td>(0/3)</td>
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<tr>
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<td></td>
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<tr>
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*Papaverine caused relaxation of previously contracted tissue.
†Recorded as percent positive responses.
‡Number of positive responses per number of trials on different tissues.
was applied immediately after surgery and the animals were followed at 1, 3, and 7 days without administration of further medication until they were killed 3 to 4 wk after wounding. By 4 hr after wounding, a fibrin clot had formed within the wound and the anterior chamber had reformed. No eye was lost to infection. There was no case of lens or iris adhesion or incarceration in the wound at the time of death, and all wounds were noted to heal without vascular ingrowth as evaluated using a handheld 10 diopter lens. The contralateral unwounded eye served as a control.

At the time of death, a 3 X 8 mm strip of cornea, excluding the limbus and passing through the central cornea, was excised from each eye. In the case of wounded corneas, the width of each strip was trimmed to include the lateral margins of the wound. As previously described by Sommers and Ryan, each specimen was then placed in buffered, ionically balanced Tyrode’s solution (pH 7.4) at 34–36°C for transport. Within 5 min after excision, the specimen was suspended lengthwise by 6-0 silk suture on a microforce transducer. Specimens were then immersed and relaxed by application of 500 mg of tension. Equilibration of the system was indicated by flattening of the tissue relaxation curve, as monitored through a dynagauge and recorded graphically by a DC linear amplifier and penwriter. Following equilibration, a 75-μl aliquot of the test solution was added to the Tyrode’s bath.

Pharmacologic agents and their test concentrations included serotonin (5-HT) 0.1 mg/ml, norepinephrine (NE) 0.2 mg/ml, epinephrine (E) 0.2 mg/ml, acetylcholine (ACh) 0.5 mg/ml, histamine (H) 0.2 mg/ml, vasopressin (V) 75 mIU, and papaverine (P) 0.2 mg/ml. (Papaverine has been shown to cause relaxation of tissues previously stimulated to contract by other agents. All agents were prepared fresh each day. The concentration of test reagents was selected based on previous reports of contractile properties of granulation tissue. Controls, including normal cornea and iris dilator and constrictor muscles (six specimens each), were tested in a manner identical to that of the corneal wounds.

Tissue responses to each agent were recorded graphically as described above. Following each trial the bath solution was removed, the tissue specimen was rinsed, and the bath solution was replaced with fresh Tyrode’s. Each tissue was re-equilibrated as indicated by re-establishment of a graphically stable baseline. During each experiment, the contractile response of four wounds or control specimens to four pharmacologic agents (A-D) in addition to papaverine were tested. In order to eliminate any sample bias and to allow for multiple testing of agents on a single specimen, the order of exposure to each agent was varied during each run such that specimen 1 received separately agents...
Fig. 3. Response of iris constrictor muscle to epinephrine. Note rapid contraction in response to addition of epinephrine, followed by prompt relaxation of tissue without further pharmacologic intervention (asterisk marks time E was added to bath).

ABCD; specimen 2, BCDA; specimen 3, CDAB; and specimen 4, DABC. Negative contractile responses were confirmed by testing for specimen viability as indicated by a positive response to an agent following the negative response. If the specimen no longer exhibited a positive contractile response, then the negative response to the previous agent was not counted.

**Results.** The percent positive responses to each pharmacologic agent is tabulated in Table 1 according to each tissue group tested. Of the 17 corneal wounds, 93%, 80%, and 91% contracted when exposed to 5-HT, NE, or E respectively. Positive responses to these agents were characterized by slow contraction, reaching a maximum of 20–100 mg of force (Fig. 1); peak responses were obtained within 5–30 minutes and persisted for several hours. The magnitude of each response was noted to vary considerably between specimens (Fig. 2). Normal cornea failed to contract in response to any agent tested, while avascular corneal wound specimens failed to respond to V, H, ACh, and BSS (P < 0.5) (Table 1). All iris muscle specimens contracted to ACh exhibiting peak responses of 30–60 mg of force within seconds and decaying over the following 10–60 min (Fig. 3). This response was unlike that of the corneal wounds, all of which failed to respond to ACh (P < 0.005). Exposure of all contracted specimens to papaverine resulted in tissue relaxation (Figs. 1, 2).

**Discussion.** The contractile behavior of avascular corneal wounds in response to selected pharmacologic agents shows characteristics similar to those previously reported for granulation tissue. These reports attributed the generation of contractile force to the action of a specific cell, the myofibroblast. Myofibroblasts appear to exhibit features of both fibroblasts and smooth muscle cells including: physiologically, in vitro contractility in response to pharmacologic agents and, morphologically, convoluted nuclei, abundant rough endoplasmic reticulum, intracytoplasmic microfilaments, basal lamina, cell–cell and cell–matrix attachments, and positive reaction to antismooth muscle antibodies. These cells have been described in other ocular wound healing processes, particularly traction retinal detachment, but have not previously been demonstrated in nonvascularized wounds. The physiologic identification of the myofibroblasts described here has been verified morphologically by light and electron microscopy, as well as by immunofluorescent techniques. These physiologic and histopathologic correlations suggest that corneal wound contractility in vitro is due to the presence of myofibroblast-like cells.

The presence of pharmacologically responsive contractile elements such as myofibroblasts in corneal wounds may have important clinical implications with regard to corneal wound healing. In particular, one of the remaining difficulties with radial keratotomy is the unpredictability of the final refractive result. This may be attributed in part to varying degrees of loss of corneal flattening resulting from contraction of the radial scars. Although further study is necessary to document the presence of contractile processes in nonperforating radial keratotomy wounds, if shown to be present, it may then be possible in the future to prevent the loss of corneal flattening. This may be accomplished by the application of appropriate pharmacologic agents that have a direct and specific effect on the contractile process within a defined postoperative interval. The resultant ability of the clinician to “fine-tune” radial keratotomy would represent a valuable tool in the management of these patients.

**Key words:** wound contraction, myofibroblasts, pharmacologic response, cornea, radial keratotomy

From the Department of Ophthalmology, University of Southern California School of Medicine and Estelle Doheny Eye Foundation, Los Angeles, California. Supported in part by a grant from Research
References