Hyperthermic Treatment of Rabbit Corneas

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Well defined heat doses (temperature × time) were applied to normal rabbit corneas in an effort to determine thermal tolerance, and to examine the effects of heat on this tissue. A purely conductive heater was chosen to minimize intraocular penetration, and avoid findings attributable to nonthermal effects of inductive sources. The etched element heater was sewn to 38 rabbit corneas. Thirty-six were treated to temperatures of 38, 45, 52 and/or 59 degrees centigrade for durations of 5, 15, or 45 min. Three eyes were treated at each time-temperature interval and sacrificed at either time 0, 1 day or 1 week follow-up. Histologic examinations were performed on all corneas. A corneal haze was first noted at 45°C × 45 minutes × 1 day follow-up. This correlated with a mild stromal edema on light microscopy. Higher thermal doses produced a spectrum of damage, with complete destruction of all keratocytes and endothelial cells at 59°C × 45 min. At levels greater than 45°C × 45 min, heat damage was noted to be increased at 24 hr follow-up. Some recovery was noted by 1 week follow-up, with the exception of the 59°C × 15 or 45 min groups. These two heat doses induced a drop-out of cellular elements with evidence of disintegration and fragmentation of collagen fibrils. Conductive heating of up to 45°C × 15 min appeared well tolerated by normal rabbit corneas. Invest Ophthalmol Vis Sci 30:1778–1783, 1989

High-level heating of corneal tissue has been investigated for the treatment of keratoconus, mycotic infections and to change the corneal refractive power.1-5 Unfortunately these treatments have been associated with destruction of Bowman's membrane, recurrent erosion syndrome, marked degeneration of keratocytes, stromal necrosis, stromal scarification, endothelial damage and bullous keratopathy.6-9

Low-level heating (hyperthermia) has been proven to be effective in the treatment of malignant neoplasms, while certain viruses, fungi and bacteria are known to be particularly heat-sensitive.10-17 It may be reasonable to consider hyperthermia as a potential adjunct to current therapy of corneal ulcers and conjunctival neoplasia involving the cornea.

In experiments with an etched element conductive heater, we have shown that up to 47°C × 1 hr was well tolerated by rabbit sclera.18 Though higher temperatures (up to 55°C) were noted to leave the sclera structurally intact, scleral damage, choroidal effusions and retinal degeneration and gliosis were noted.19

In an effort to preserve underlying tissues, we chose to employ a purely conductive heat source (etched element heater), because it merely deposited heat on the surface of the cornea. Therefore, all intracorneal heating would be a result of passive diffusion.18-20 This contrasts to inductive heaters (eg, microwave, ultrasound, radiofrequency or direct current) which induce heating within tissues.21

This study was conducted to determine the effects of specific heat doses (temperature × time) on normal rabbit corneas. The spectrum of thermal doses studied encompass both the range commonly used in the treatment of cancer (42–45°C) up to levels known to cause scleral collagen breakdown (60°C).11,19,22

Materials and Methods

This investigation was conducted in adherence to the ARVO Resolution on the Use of Animals in Research. The etched element heaters used in this study were thin laminated devices constructed to make the heating surface 1.0 cm in diameter and less than 1.0 mm in thickness. The etched-foil (75 Ohm) element was encased in a clear amber polymide base film (insulation) with a 4.0 mm free margin at the periphery, through which sutures were placed for attachment to the cornea (Fig. 1).
A copper–constantan thermocouple thermometer was affixed to the center of the etched element heater and was used to regulate corneal temperature. A direct-current supply was required to power the heater and a digital thermosensor was used to continuously monitor epicorneal temperatures during treatment.

Heater Placement

Thirty-eight rabbits were anesthetized with a combination of ketamine hydrochloride (20 mg/kg) and xylazine hydrochloride (20 mg/kg). Intramuscular injection provided approximately 1 hr of sedation. Topical anesthesia 0.5% proparacaine hydrochloride solution was used prior to insertion of the wire eyelid speculum.

The etched element heater was secured to the cornea by four 7-0 nylon sutures passed through the clear amber polyimide film and through the conjunctiva and sclera just posterior to the corneoscleral limbus at approximately the 3, 6, 9 and 12 o’clock positions. Symmetry provided even contact on the center of the cornea (Fig. 1). Complete contact of the entire thermocouple-bearing surface of the heater to the cornea was required to insure an even temperature distribution and accurate temperature measurements.

Hyperthermic Treatment

Hyperthermia was induced in a circular area defined by the shape of the etched element heater. Corneal surface temperatures were continuously monitored and recorded. Desired temperature values were reached within 1 min of onset of thermal treatment.

Baseline temperatures ranged from 33.2°C to 36.4°C for the 38 corneas. Three eyes were treated at each time–temperature interval at values of 38, 45, 52, or 59°C for 5, 15, or 45 min durations. Then one cornea at each data point was removed at either time 0, 24 hr or 1 week after treatment. External photographs of the 12 animals followed for 1 week after treatment were obtained at time 0, 24 hr and at 1 week follow-up.

All 36 treated and two sham-treated corneal buttons were removed with an 8 mm trephine, bisected and placed into solutions for preservation. The corneas for light microscopic evaluation were immediately fixed in cold buffered 10% formaldehyde. Following alcohol dehydration, the corneas were embedded in paraffin. The slides were stained with hematoxylin and eosin and evaluated blindly. The corneas for ultrastructural evaluation were immediately fixed in cold, buffered 2% glutaraldehyde, post-fixed in 1% osmium tetroxide, and embedded in epoxy resin for electron microscopy. All specimens underwent histologic examination while ultrastructural examinations were also performed on four corneal specimens (sham, 45°C × 45 min, 52°C × 15 min and 59°C × 15 min).

Results

The nine corneas heated to 38°C at all time intervals and all durations, the six corneas treated to 45°C × 5 min and × 15 min, and the two sham-treated corneas at all durations of follow-up (time 0, 24 hr and 1 week) were found to be grossly and histologically normal.

The first definite change occurred at 45°C × 45 min × 1 day. This cornea, which appeared cloudy on gross examination, exhibited mild stromal edema on light microscopy (Fig. 2).

At 52°C × 5 min × time 0 cornea was grossly normal but showed subepithelial stromal changes on light microscopy. Stromal edema increased at 1 day post-treatment with evidence of collagen disorganization. Only slight stromal edema was evident after 1 week follow-up.

At 52°C × 15 min × 1 day, mild corneal edema was clinically apparent. Light microscopy revealed epithelial loss and massive stromal edema, with intense collagen changes characterized by disorganization and vacuole formation within the anterior stroma. This thermal dose produced the first signs of keratocyte damage (crenation). Less overall damage was evident at 1 week follow-up, which may represent some recovery.

At 52°C × 45 min × 1 day, gross clouding of the cornea was evident. On light microscopy stromal edema and vacuole formation were again evident (Fig. 3). The earliest observation that stromal collagen fibrils were being teased apart was confirmed on ultrastructural studies. At 1 week follow-up, pyknotic nuclei and partial destruction of Bowman’s membrane were noted.

The cornea treated at 59°C × 5 min × time 0 showed a gross corneal haze with stromal edema, intrastromal vacuole formation and plump keratocyte nuclei on light microscopy (Fig. 4). Twenty-four hours later, more nuclear degeneration and stromal disorganization were present. At 1 week there were indications of possible repair, but stromal cellular damage persisted.

At 59°C × 15 min × 1 day, light microscopy revealed massive stromal edema with total cellular drop-out and loss of endothelial cells. Electron microscopy confirmed the general disorganization of collagen fibrils, and more dramatically demonstrated the fracturing of collagen bundles (Fig. 5). The 1 week follow-up specimen demonstrated cellular drop-out, though a few pyknotic nuclei had persisted.
For the rabbit corneas treated at 59°C × 45 min, corneal clouding and mydriasis persisted to 1 week follow-up (Fig. 6). Light microscopy revealed severe degeneration of all structures, near-total necrosis without perforation. The cornea appeared homogeneously degenerated (Fig. 6).

Histopathologic changes noted in this study included corneal edema, a spectrum of findings consistent with coagulative necrosis of the stromal collagen, and damage or loss of cellular elements (Table 1). Our findings suggest that up to 45°C × 15 min at all time durations was well tolerated by normal rabbit
corneas, 52°C was a transitional temperature for keratocyte damage, and no corneal perforations were noted at any time-temperature interval or follow-up period.

Discussion

As illustrated by this study, a thermal dose can be defined by a time-temperature relationship. If the dose is large enough any tissue or cell can be destroyed by a physical agent such as heat. We have produced a spectrum of thermal damage at varying dose levels in corneal tissue.

Relatively high thermal doses appeared well tolerated by the rabbit cornea; these doses are in a range known to be useful in the adjuvant therapy of cutaneous and choroidal melanoma.23-26 Therefore heat treatment of conjunctival neoplasia (eg, melanoma, squamous carcinoma) that involve the cornea may be possible. These diseases are known for their high incidence of recurrence and may benefit from adjuvant heat therapy.27-29

Corneal infections due to fungi and acanthamoeba are increasingly common and known to be difficult to treat.30,31 Since high-level heating over short periods of time has been used to destroy bacteria, viruses and fungi, low-level heating over extended periods of time may also inhibit or destroy these organisms.13-17

For thermal doses up to 52°C × 5 min, there was no evidence of histologic damage at the 1 week follow-up examination. This may represent the type of recovery noted in our earlier experiments with 1 month follow-up.20 We believe this may be related to the remarkable regenerative capacity of rabbit corneas. In this study, it is important to note that there was no evidence of permanent stromal collagen disorganization or keratocyte damage at up to and including 52°C × 5 min, and that corneal perforation did not occur at any of the time-temperature regimens studied.

We conclude that low-level conductive heating of normal rabbit corneas produced a spectrum of thermal damage related to the temperature, duration of heating and the follow-up period. Further study will
Table 1. Summary of histological findings on light microscopy

<table>
<thead>
<tr>
<th>Temp (°C)</th>
<th>Time (min)</th>
<th>Time 0</th>
<th>1 day</th>
<th>1 week</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
<td>05</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>38</td>
<td>15</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>38</td>
<td>45</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>45</td>
<td>05</td>
<td>Normal</td>
<td>Mild stromal edema</td>
<td>Mild stromal edema</td>
</tr>
<tr>
<td>45</td>
<td>15</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
</tr>
<tr>
<td>45</td>
<td>45</td>
<td>Normal</td>
<td>Stromal edema</td>
<td>Normal</td>
</tr>
<tr>
<td>52</td>
<td>05</td>
<td>Subepithelial collagen change, edema</td>
<td>Disorganized stroma with massive edema, anterior stromal vacuolization</td>
<td>Diffuse stromal collagen smudging, crenation of nuclei</td>
</tr>
<tr>
<td>52</td>
<td>15</td>
<td>Superficial stromal collagen changes, generalized edema</td>
<td>Intense stromal destruction, edema-vacuolization-disorganization</td>
<td>Intense nuclear degeneration, partial destruction of Bowman's membrane</td>
</tr>
<tr>
<td>52</td>
<td>45</td>
<td>Diffuse stromal collagen changes, generalized edema</td>
<td>Loss of keratocytes from anterior stroma, collagen fibers appear teased apart</td>
<td>Stromal cellular dropout, Descemet folds, edema</td>
</tr>
<tr>
<td>59</td>
<td>05</td>
<td>Vacuolated, rounded nuclei, diffuse edema</td>
<td>Nuclear degeneration, generalized stromal disorganization</td>
<td>Few pyknotic stromal cells, intense stromal disorganization</td>
</tr>
<tr>
<td>59</td>
<td>15</td>
<td>Moderate stromal collagen smudging, cellular elements present in all layers</td>
<td>Total cellular drop-out, no evidence of epithelial, stromal or endothelial cells</td>
<td>Severe degeneration of all structures, total necrosis</td>
</tr>
<tr>
<td>59</td>
<td>45</td>
<td>Stromal collagen degeneration, loss of epithelium, rounded nuclei with disruption of Descemet's membrane</td>
<td>Total loss of cellular elements, massive edema and disorganization of stromal collagen</td>
<td>Severe degeneration of all structures, total necrosis</td>
</tr>
</tbody>
</table>

be required to determine the potential role of low-level heating in treatment of corneal diseases.32–34

**Key words:** cornea, heat, conductive, hyperthermia, plaque

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**References**

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