Cyclosporine Inhibits Apoptosis in Experimental Murine Xerophthalmia Conjunctival Epithelium

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Summary[1] This study examined the inhibitory effect of topical cyclosporine (CsA) treatment on conjunctival epithelial apoptosis in a murine model of xerophthalmia. Dry eye was induced in 3 groups of C57BL6 mice by subcutaneous injection of scopolamine (t.i.d) and exposure to an air draft and low-humidity environment for 16 h each day for 12 days. The dry eye control group received no topical treatment; another group received 1 µL of 0.05 % CsA topically (t.i.d, dry eye+CsA); and the third group received 1 µL of the castor oil vehicle of CsA topicaly (t.i.d, dry eye + vehicle). Normal mice were used as untreated controls. Twelve days later, the mice were killed, and their conjunctivais were excised. The number of the conjunctival goblet cells was counted in tissue sections stained with periodic acid Schiff (PAS) reagent. Their conjunctiva epithelium had been investigated by immuno-histochemical staining to detect the goblet cells and the expression of Caspase-3, Bax and bcl-2. Our results showed that compared with dry eye control and dry eye mice + vehicle groups, the number of conjunctival epithelial goblet cells was significantly greater in the untreated controls and dry eye mice receiving CsA (P < 0.01 for both groups). There was no significant difference in the number of conjunctival epithelial goblet cells between the dry eye control and dry eye+vehicle group. It was also true of the number of conjunctival epithelial goblet cells when comparison was made between the normal group and the dry eye+CsA group. Expressions of Caspas-3 and Bax were increased and ex-pression of bcl-2 was decreased in conjunctival epithelial cells in dry eye control and dry eye mice+vehicle groups. There was a significant positive correlation between goblet cell number and the number of cells that expressed bcl-2, and a negative correlation between goblet cells and Caspase-3 and Bax expression. It is concluded that the topical use of CsA could significantly reduce conjuncti-val epithelial apoptosis and protect goblet cell against the loss in experimental murine xerophathal-mia. Inhibition of apoptosis appears to be a key mechanism responsible for the therapeutic effect of CsA on xerophthalmia.

Key words[1] xerophthalmia; cyclosporine (CsA); apoptosis; gene
DOI 10.1007/s 11596-006-0424-8

Dysfunction of the functional units of surface-lacrimal gland leads to tear film instability and the ocular surface epithelial disease called xerophthalmia, which results in eye irritation and vision impairment. The pathological mechanisms underlying xerophthalmia remain unknown, but the study by Yeh et al showed that accelerated apoptosis of the ocular surface epithelia may play a role in the process[1]. Cyclosporine A (CsA) is the first FDA-approved drug used for the treatment of xerophthalmia, and its possible mechanism for the amelioration of eye surface epithelium disease lies in the inhibition of apoptosis[2]. This study is to determine if topical CsA treatment for xerophthalmia can inhibit apoptosis of the ocular surface epithelia in response to experimentally induced dry eye in mice.

1 MATERIALS AND METHODS

1.1 Experimental Animals

Forty C57BL6 mice of both sexes, aged 8 weeks, weighing (20±2) g, were provided by Organ Transplantation Center of Wuhan Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China[1] aged 8 weeks, weighing of both sexes were used in the study.

1.2 Procurement and Preparation of Specimens

1.2.1 Establishment of Conjunctival Xerosis Model

Thirty C57BL6 mice aged 8 weeks were hypodermically injected scopolamine at a dose of 0.2 mL/mg (t.i.d) for 12 days.

1.2.2 Grouping and Treatment

The 30 mice were divided into 3 groups, with each group having 10 animals. The 10 mice in dry-eye control group received no eye drop; 10 mice in dry-eye+CsA group were given 1 µL 0.05 % ciclosporin A (CsA) emulsion (Pharmacy of Tongji Hospital, Wuhan, China) 3 times a day for a time of 12 days. Ten mice in dry-eye+bearer group were administered CsA agent 1 µL castor oil (Tianjin Standard Technology Co., Ltd., China) 3 times a day for a time period of 12 days. Another 10 mice served as normal group and received no treatment.

1.3 Harvesting and Treatment of Specimen

After above-mentioned treatment for 12 d, the mice of the 4 groups were sacrificed and the tissues of the
conjunctiva of both eyes were taken and fixed by 4% paraform, and subjected to routine paraffin imbedding.

1.4 Detection Techniques

1.4.1 PAS Staining Sections were stained with periodic acid Schiff (PAS) reagent to evaluate the conjunctival goblet cell density. The caliciform cells in the 4 groups were counted under high power microscope (400 folds) and the average cell number in each high-power field was calculated.

1.4.2 Immunohistochemistry Sections adjacent to those used for PAS staining were used for immunohistochemical staining for activated caspase-3, Bax or bcl-2. SABC kit (Wuhan Boster Bioengineering Ltd., China) was employed. The detailed procedures were done following the instructions of the kit. The polyclonal goat anti-mouse caspase-3, Bax and bcl-2 primary antibody (PharMingen, USA) were diluted to 1:100. The total number of cells positive for the immunohistochemical staining in 1000 conjunctival epithelial cells was calculated.

1.5 Cell Counting and Statistical Analysis

The total number of goblet cells in 5 high power fields were counted and the average number per field was calculated. The total number of cells positive for the immunohistochemical staining in 1000 conjunctival epithelial cells was calculated. The data were expressed as X±s and SPPS 12.0 package was used for the statistical analysis (SNK method).

2 RESULTS

2.1 Findings after Treatment

Twelve days after the treatment, in the dry eye control group and dry-eye+vehicle group, the hair of the mice was coarse; their cornea was dry and lacked luster. Fluorescein staining and rose Bengal staining revealed scattered spotty ulcer. Rose Bengal staining and breakup time (BUT) were measured and the Schirmer’s test was conducted. There were significant differences between dry-eye control group and dry-eye+vehicle group and between normal group and dry-eye+CsA group (P<0.01, table 1).

Table 1 Results of Rose Bengal, BUT and chirmer test (X±s)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Rose Bengal</th>
<th>BUT(s)</th>
<th>ST (mm)</th>
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<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>26.18±36.58&lt;sup&gt;+&lt;/sup&gt;</td>
<td>25.34±5.69&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry eye</td>
<td>15.23±2.76</td>
<td>6.94±2.41</td>
<td>7.36±1.42</td>
</tr>
<tr>
<td>Dry+vehicle</td>
<td>11.65±1.86</td>
<td>8.59±1.27</td>
<td>8.29±3.06</td>
</tr>
<tr>
<td>Dry+CsA</td>
<td>1.87±0.48&lt;sup&gt;+&lt;/sup&gt;</td>
<td>22.08±2.73&lt;sup&gt;+&lt;/sup&gt;</td>
<td>21.09±4.38&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*, † P<0.001</sup> as compared with dry eye and dry eye+vehicle group; ††<sup>+</sup> <sup>† P<0.01</sup> as compared with dry eye and dry eye+vehicle group

2.2 PAS Staining of Goblet Cells

There were mainly two kinds of cells in normal group and dry eye+CsA group. One was global cells, which were big and abundant. The nuclei were round and stained blue-purple and was located near one end of cells. The cytoplasm was abundant and contained plenty of mucus and it was strongly PAS-positive and red-purple in color. The other kind was epithelia, which were small and even in size. They were closely and densely arranged in patches and their nuclei were round and blue in color and tended to lie in the middle of the nucleus. Their cytoplasm was bluish or reddish. In dry eye group and dry-eye+vehicle group, conjunctival epithelial cells were irregularly arranged and had few goblet cells few PAS-positive substances. Compared with dry eye control group and dry-eye+vehicle groups, number of conjunctival epithelial goblet cells was significantly greater in the normal group and dry eye +CsA group (P<0.01 for both, table 2).

Table 2 Goblet cells and expression of the related genes in the conjunctival epithelium of each group (X±s)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Goblet cells (n)</th>
<th>Caspase-3/1000 cell</th>
<th>Bax/1000 cells</th>
<th>Bcl-2/1000 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>52.2±2.3&lt;sup&gt;+&lt;/sup&gt;</td>
<td>73.52±2.5&lt;sup&gt;+&lt;/sup&gt;</td>
<td>52.47±2.5&lt;sup&gt;+&lt;/sup&gt;</td>
<td>253.18±2.5&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dry eye</td>
<td>27.3±2.3</td>
<td>303.35±2.3</td>
<td>77.49±2.3</td>
<td>56.39±2.3</td>
</tr>
<tr>
<td>Dry+vehicle</td>
<td>25.0±1.8</td>
<td>289±1.8</td>
<td>82.49±1.8</td>
<td>35.69±1.8</td>
</tr>
<tr>
<td>Dry+CsA</td>
<td>50.8±2.8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>56.39±2.8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>247.35±2.8&lt;sup&gt;+&lt;/sup&gt;</td>
<td>23.38±2.8&lt;sup&gt;+&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>*, † P<0.001</sup> as compared with dry eye and dry eye+vehicle group

2.3 Expressions of Apoptosis Relative Gene Protein

Caspase-3, Bax and bcl-2

The expressions of caspase-3 and Bax could be seen in the conjunctival epithelial cells of dry-eye group and dry-eye+vehicle group, the expression was obviously higher than those of the normal group and the dry-eye+CsA group. The positive reaction products were brown on color and mainly found in cytoplasm. The difference between them was statistically significant (P<0.01, table 2).

The expression of bcl-2 could be seen in the conjunctival epithelial cells of normal group and dry-eye+CsA group, and they were obviously stronger than those of dry-eye group and dry-eye+vehicle group (P<0.01). The positive reaction products were brown I color and mainly found in the cytoplasm (table 2).

2.4 Correlative Analysis of Goblet Cell Population and Relative Gene Expression

The expression of caspase-3 and Bax in conjunctival epithelial cells was negatively correlated with the number of goblet cells (normal group: r=-0.79, -0.86; P<0.05; dry-eye control group: r=-0.72, -0.83; P<0.05; dry-eye+vehicle group: r=-0.69, -0.77; dry-eye+CsA group: r=-0.73, -0.71; P<0.05). The expression of bcl-2 in conjunctival epithelial cells had a positive correlation with...