**ABSTRACT**

**Purpose:** The purpose of this study was to evaluate a dry eye murine model using simplified methods for testing the efficacy of potential anti-dry eye compounds as novel dry eye treatments. Dry eye in this model was induced by injection of rimabotulinum toxin B (BTX-B) into the lacrimal glands.

**Methods:** Thirty-six (36) CBA/J mice were used in the study and were divided equally into blank, saline, and BTX-B groups. Twelve (12) animals were injected with saline or with BTX-B into both lacrimal glands on Day 0. Half of the animals in each group were sacrificed at two weeks or four weeks after the lacrimal gland injections. During the study, the following parameters were measured on all animals at pre-study, twice weekly and at pre-sacrifice: Tear production measurement (TPM); corneal front membrane fluorescein break-up time (FBUT); and corneal fluorescein staining (CFS). TPM was measured with standardized phenol red-impregnated cotton threads (mm). FBUT was recorded (seconds) and CFS was scored (grading 0-4) with a SH-Lamp under blue cobalt light. Histopathological analysis was performed after staining with hematoxylin and eosin (H & E). All animals were clinically observed daily and were weighed at pre-study and at pre-sacrifice.

**Results:** The average TPM of animals in the BTX-B group was 1.7 mm ± 0.3 mm at two weeks and 1.4 ± 0.4 mm at four weeks. There was a statistically significant time-dependent decrease in the average TPM of animals in the BTX-B group compared to the that in the blank group (3.1 ± 0.6 mm at two weeks and 3.7 ± 0.8 mm at four weeks) and in the saline group (3.4 ± 0.8 mm at two weeks and 3.6 ± 0.7 mm at four weeks) (p < 0.05). The average FBUT of animals in the BTX-B group was 6.0 ± 0.9 seconds at two weeks and 4.2 ± 0.7 seconds at four weeks. There was a statistically significant time-dependent decrease in the average FBUT of animals in the BTX-B group compared to that in the animals in the blank group (9.4 ± 1.0 seconds at two weeks and 10.0 ± 1.1 seconds at four weeks) and in the saline group (9.8 ± 1.1 seconds at two weeks and 9.5 ± 1.4 seconds at four weeks) (p < 0.05). In the BTX-B group, the average CFS grades were 2.3 ± 0.9 at two weeks and 3.3 ± 0.5 at four weeks. There was a statistically significant time-dependent increase in the average CFS grades in the BTX-B group compared to those in the blank or the saline group (0.2 ± 0.4 at two weeks and 0.2 ± 0.4 at four weeks) (p < 0.05). Histopathological analysis revealed no remarkable differences in any group at either the time-point of two or four weeks. However, a small acinar focal area was found in the right lacrimal gland of one animal in the BTX-B group at the time-point of four weeks.

**Conclusions:** The dry eye mouse model which was induced by BTX-B injections into the lacrimal glands was evaluated based on ocular examinations by TPM, FBUT, CFS, and histopathology. BTX-B injections into the lacrimal glands of mice decreased TPM and FBUT, but increased CFS. These results validate that a dry eye model in mice was successfully created. This model can be successfully used to test the efficacy of potential anti-dry eye compounds.

**METHODS AND MATERIALS**

**Animals**

36 CBA/J mice: Female; Body weight: Approx. 20 grams; Age: At least 6 weeks old.

**Time-point (weeks)**

<table>
<thead>
<tr>
<th>Group</th>
<th>Time-point (weeks)</th>
<th>Blank control</th>
<th>Saline control</th>
<th>BTX-B Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 (14 days)</td>
<td></td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>4 (28 days)</td>
<td></td>
<td>6</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>12</td>
<td>12</td>
<td>12</td>
</tr>
</tbody>
</table>

**Dry Eye Model Creation**

Dry eye was induced by injection of 50 μL of rimabotulinum Toxin B (BTX-B) (20 mU/mL) into the lacrimal glands on Day 0.

**Lacrimal Glands Tear Production Measurement (TPM)**

The fluorescein tear break-up time (FBUT) is an indirect measure of tear film stability. A break-up time of ≤ 10 seconds indicates dryness.

**Fluorescein Tear Film Break-up Time (FBUT)**

The fluorescein tear film break-up time (FBUT) is an indirect measure of tear film stability. A break-up time of ≤ 10 seconds indicates dryness.

**Corneal Fluorescein Staining**

Grading:

- 0: ≤ 0.5
- 1: ≤ 1/2
- 2: ≤ 1/4
- 3: ≤ 1/8
- 4: ≤ 1/16

**Corneal Fluorescein Staining Score**

Day after Saline or BTX-B Injection (28 days)

**SUMMARY**

Clinical observations: The dry eye mouse model which was induced by BTX-B injection into the lacrimal glands was evaluated based on ocular examinations by TPM, FBUT, CFS, and histopathology. The TPM and FBUT decreased and CFS increased in the BTX-B group compared to blank or saline group (p < 0.05) at 2 or 4 weeks after BTX-B injection. Histopathological analysis: There were no differences among groups at 2 or 4 weeks after BTX-B injection other than a small acinus focal area found in the right lacrimal gland of one animal in the BTX-B group 4 weeks after the BTX-B injection. These results validate that a dry eye model in mice was successfully created. This model can be successfully used to test the efficacy of potential anti-dry eye compounds.

**RESULTS**

**Day 0**

TPM

**Day 14**

TPM

**Day 28**

TPM

**RESULTS**

**Mean Tear Production Measurements**

**Corneal Fluorescein Staining Score**

Day after Saline or BTX-B Injection (14 days)

**Fluorescein Tear Break-Up Time (FBUT)**

Day after Saline or BTX-B Injection (14 days)

**Corneal Fluorescein Staining Score**

Day after Saline or BTX-B Injection (28 days)

**REFERENCES**
