Use of Environmental Conditions to Enhance the Induction of Keratoconjunctivitis Sicca in Mice, Rabbits, and Dogs

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Purpose
Dry eye syndrome, or keratoconjunctivitis sicca (KCS), affects millions of people worldwide. The dry eye condition causes ocular irritation, blurred vision, ocular surface diseases, and the risk of corneal ulcerations. Complementary animal models of KCS including rodents, rabbits, and dogs were developed to evaluate new therapies. The utilization of environmental parameters was assessed for its impact in helping accelerate the KCS condition.

Methods

**Mouse:** CD-1 mice were housed under arid conditions (~20% humidity) in either a standard rodent cage or an airflow chamber (cage fitted with a modified airflow system) and administered 50µL of 1% atropine topically into both eyes every other day for 8 days (N=5/group). A control group (N=2) was housed under standard conditions. Tear production and corneal damage were assessed using modified Schirmer tear tests and fluorescein staining on Days 0, 3, 5, and 8.

**Rabbit:** Female New Zealand White rabbits (N=10) were housed at ~20% humidity with increased airflow and administered 50µL of 1% atropine topically into both eyes twice daily (BID). Tear production was assessed using Schirmer tear tests and fluorescein tear break-up tests once weekly.

**Dog:** Female Beagle dogs were housed at ~20% humidity with increased airflow and administered 50µL of either 1% atropine (N=5) or 0.08% benzalkonium chloride (BAC) (N=5) topically into both eyes 3x daily for 43 days. Starting on Day 21, animals received 50µL of either RESTASIS® (N=5) or vehicle (N=5) topically into both eyes twice daily (BID). Tear production was assessed using Schirmer tear tests and fluorescein tear break-up tests once weekly.

**Results**

**Mouse:** Topical ocular atropine in combination with an environmental air flow chamber showed a decrease in tear production in the controlled environment room when compared to the control group (Group 1). Figures 1 and 2. Group 2 mice were housed in the controlled environment room. In the airflow chamber, mice were observed to have the largest decrease in tear production and showed uptake of the fluorescein stain, indicating a compromised corneal epithelium cell layer (Group 3), Table 1.

**Rabbit:** Tear breakup times decreased during the KCS induction phase for all groups which is indicative of the dry eye condition. Tear breakup times increased for the rabbits treated with RESTASIS® during the treatment phase (Figure 3). During the KCS induction phase, a decrease in tear production was observed with decreased Schirmer tear values. There was an observed increase in tear production in animals treated with RESTASIS® compared to the placebo treated group.

**Dog:** Schirmer Tear Test findings suggested that tear production was reduced in atropine-induced animals in combination with the airflow chamber and controlled environment room (Figure 4). The environmental conditioned room appears to accelerate KCS when compared to those only treated with atropine in normal room conditions. Fluorescein staining findings reflected corneal damage developing after the induction phase (Figure 5). This damage appeared to be attenuated in animals receiving RESTASIS® treatment. There was a significant main effect of Treatment when compared to the untreated control (p < 0.05). Post hoc testing found that the differences between RESTASIS® and vehicle-treated groups reached significance in Week 6 (p < 0.05).

Shimmer Tear Test values were not reduced when treated with the 0.08% BAC (Figure 6). The BAC appeared to be more of an irritant than reducing tear formation. In addition, BAC induced only corneal damage. No effect of RESTASIS® treatment on either tear production or corneal damage was found in BAC-induced animals.

**Conclusions**
Environmental parameters can be used to enhance the keratoconjunctivitis sicca condition compared with the use of topical agents alone. Atropine in combination with environmental conditions led to both reduced tear production and increased corneal damage. This enables the development of sensitive preclinical models to test the efficacy of novel treatment therapies.

**References:**