Dry eye: Two experimental rodent models for drug development.

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Purpose

Dry eye syndrome is a relatively common disease with multifactorial causes and can afflict anyone of any age. It is thus necessary to have an experimental model to test and select therapeutic candidates for this disease.
The classification[1] differentiates dry eye by hyposecretion syndromes and dry eye with tear film instability. In recent years, many discoveries have significantly changed the understanding of dry eye. The important roles played by inflammation of the ocular surface and lacrimal[2] as well as hormonal factors[3] or anomalous lacrimal and meibomian gland function are studied in animal models and patients.
Here we describe two experimental models of dry eye in which scopolamine, a tropane alkaloid drug with muscarinic antagonist effects, is employed to suppress lacrimation and induce dry eye symptoms.

Methods

The rat model

For this first model, fifteen female Lewis albino rats (180-200g) were randomized in three groups of five animals: a naive group (not induced) and two induced groups, one treated by oral administration of cyclosporine A (20mg/kg/day) and the other one by instillations of 0.9% NaCl three times daily.
Experimental dry eye was induced in rats by systemic and continuous delivery of scopolamine (20mg/day) over 21 days from osmotic pumps (2ML4 Alzet®; Charles River Laboratories, France) implanted subcutaneously on D1[4].
Rats were kept under controlled temperature (22+/-1°C), humidity (55-65%) and 12h:12h light-dark cycle (10-200 lux).

The mouse model

For this second model, thirty female C57BL/6N mice (20g) were randomized in three groups of ten animals: a naive group (not induced) and two induced groups, one treated by oral administration of cyclosporine A (20mg/kg/day) and the other one by instillations of 0.9% NaCl three times daily.
Experimental dry eye was induced by applying a transdermal scopolamine patch (0.5mg/72h) on mice that were placed in a controlled environmental chamber (CEC) (relative humidity <25%, air-flow 15l/min, temperature 20-22°C and 12h:12h light-dark cycle (10-200 lux) from D1 to D7[5].

Rodents were handled and cared for according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Food and water were available ad libitum.

Clinical Evaluations

Tear production was measured with the cotton thread test (Zone Quick, FCI Ophthalmics, USA) in the lateral cantus of the conjunctival fornix for 30 seconds.
Corneal defects were examined by slit-lamp observation using blue light after instillation of 2µl of 0.5% sodium fluorescein for rat evaluation and 0.5µl of 0.5% sodium fluorescein for mouse evaluation. Punctate staining was measured using a grading system (National Eye Institute) giving a 0-3 score to each of 5 areas of a divided cornea for a maximum score of 15.
These examinations were performed in both eyes in the rats at baseline and on D7, D14 and D21, and in the mice at baseline and on D2 and D7.

Statistical analysis

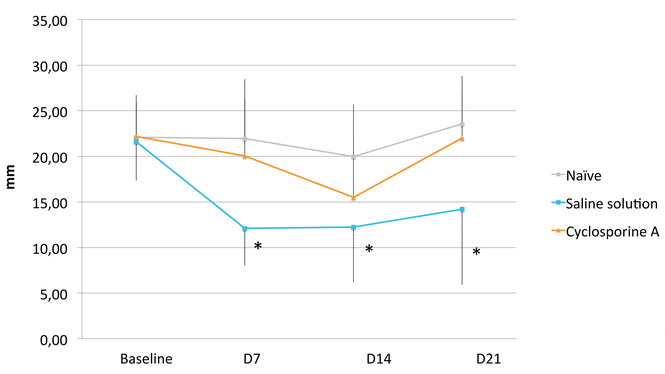
Result were expressed as mean +/- SD. Data were compared using the nonparametric Mann-Whitney statistical test. * *: $p < 0,05$*

Results

Cotton Thread Test

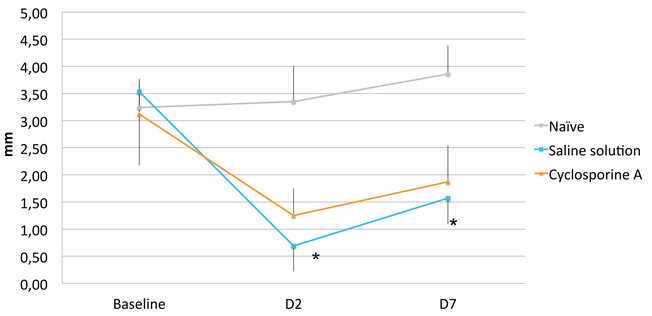
The rats induced with scopolamine and treated with saline solution showed a rapid and significant decrease of lacrimation by D7. Oral administration of cyclosporine A preserved the tear volumes. In this group, the tear volumes were close to the baseline values except on D14.

Cotton Thread Test in the Rat Model



The mice housed in the CEC and exposed to scopolamine showed a rapid and significant decrease of lacrimation by D2. Oral administration of cyclosporine A did not show a significant increase of the tear volumes.

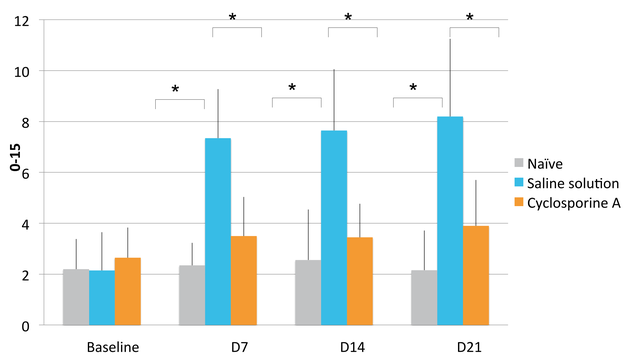
Cotton Thread Test in the Mouse Model



Fluorescein Staining

In the rat model, a relatively severe keratitis punctata was visible by D7 as illustrated by a significant increase of the score (300%) in the induced group. Oral cyclosporine A produced a marked reduction of corneal damage by D7.

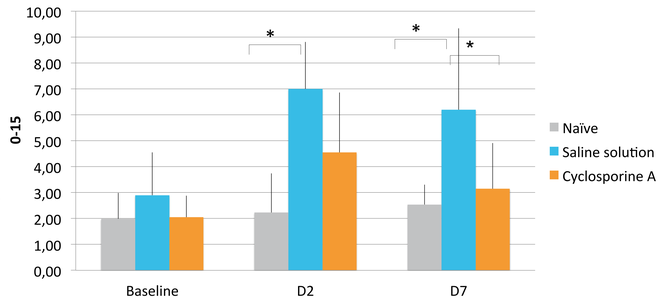
Fluorescein Staining in the Rat Model



As in the rat model, corneal defects were visible by slit-lamp on D2 and D7 in the induced mice.

Cyclosporine A effected a decrease of the keratitis punctata that was significant on D7.

Fluorescein Staining in the Mouse Model



Conclusions

Scopolamine is a good inducer for a dry eye model in rodents. The data show a rapid and significant decrease of the lacrimation and an increase of the corneal defects visible by slit-lamp evaluation. As in human disease, cyclosporine reduces clinical signs of dry eye by increasing lacrimation or decreasing corneal defects.
The two models could be used to test and select treatments for dry eye.

References

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