BILASTINE EYE DROP
ALLERGIC CONJUNCTIVITIS
Preclinical & Pharmaceutical Development
Example of public-private collaboration
What are we looking for our projects?
relay teams where different abilities can help to reach a goal.
Ocular allergy is a chronic disease that represents one of the most frequent allergic reaction and clinical problems (20-25% of the population and the incidence is increasing). This disease affects people of all ages, with no difference in sex distribution. The largest group is allergic conjunctivitis (80-90% of cases), associated with environmental allergens.

Signs and symptoms are itching, tearing, conjunctival edema, hyperemia, watery discharge, burning, photophobia and eyelid edema. Serious sequelae (e.g. eye involvement) are extremely rare in patients with conjunctivitis although the quality of life of patients may be highly affected due to the frequency and duration of the disease. Diagnostic tests in allergic conjunctivitis are based on conjunctival scrapings showing eosinophils in 80%, tear film and serum IgE levels are elevated, and tear film mast cell activity is increased (measured by immunoassay for tryptase, unique to mast cells).

Alternative topical ocular antiallergic treatments include: steroids, NSAIDs, vasoconstrictors, mast cell stabilizers or antihistamines, which act by blocking histamine H1 receptor (levocabastine, olopatadine or azelastine, have been developed for topical ocular use).

Bilastine is a new and selective histamine H1 antagonist for symptomatic treatment of allergic rhinoconjunctivitis and urticaria, developed for oral use (20 mg/day) by FAES FARMA S.A. The aim of these studies was to investigate the effect of Bilastine on experimental models of acute conjunctivitis in guinea pigs. Its activity was compared with those of Azelastine, Fluorometolone, Ketotifen, Levocabastine and Olopatadine.
Project Outline
Preclinical development

pharmaceutical development

Preclinical Pharmacology
Preclinical Toxicology

Stabilities, Industrial scale, analysis methods

Strong collaboration help getting to the clinical phases of drug development.
Eye drop pharmaceutical pre-formulation

- Developed formulation:
  - sterile solution
  - shows isotonic and pH values of lachrymal fluid.
  - shows adequate viscosity in order to extend contact time of drug with tissue.
  - no preservative, monodose.

- Pre-formulation of 0.5 mg/ml solution
- Analytical development
- Analytical method validation
- Excipient compatibility study
- Stability study of prepared formulations

Purpose

Activities
Effect of Bilastine on Experimental Conjunctivitis Model in Guinea Pig

Total Score (T) = Edema (E) + Reddening (R) + Lacrimation (L)

- 0.05% - Bilastine and competitors are active and have a similar range of action on conjunctivitis symptoms.
- 0.0125% - Bilastine has the same maximal effects.
Effect of Bilastine on Experimental Conjunctivitis Model in Guinea Pig

Bilastine eye drops: concentration-effect relationship

Total Score (T) = Edema (E) + Reddening (R) + Lacrimation (L)

- 40×10⁻⁴/0.0004% of Bilastine keeps the maximal effect. Therefore, we can consider this dose as the minimal dose with maximal effect.
Effect of Bilastine on Experimental Conjunctivitis Model in Guinea Pig

Eye drops: comparative minimal concentration with maximal effect

![Graph showing inhibition of various compounds](image)

BILASTINE AZELASTINE LEVOCABASTINE OLOPATADINE

40 x 10^{-4} mg/ml = 40 x 10^{-5} %
Effect of Bilastine on Experimental Conjunctivitis Model in Guinea Pig

Conclusions

1. Bilastine at concentrations of 0.05 %, 0.025 %, and 0.0125 %, shows a local effect similar to commercial eye drops of Azelastine (0.05 %), Levocabastine (0.05 %) and Olopatadine (0.1 %).

2. Bilastine, in a unique instillation, shows a significant activity even at a concentration 1,000 times lower (0.00005 %) than the original formulation. Moreover, protection is nearly total up to 0.00040 %.

3. As a result of the comparison at lower active concentrations, Bilastine behaviour is similar to that of Levocabastine; there is no significant difference between equal concentrations of the two products. Nevertheless, Bilastine shows higher potency than Olopatadine and Azelastine.

4. Ketotifen (0.025 %) showed an unwanted systemic effect and Fluorometholone (0.1 %) showed no activity at all in this experimental model of acute conjunctivitis.
Topical Effect on Pollen-Induced Conjunctivitis in Guinea Pig

Topical effect on pollen-induced conjunctivitis in guinea pig. Bilastine vs. commercial eye drops. Week compilation effect

<table>
<thead>
<tr>
<th>% INHIBITION vs control group</th>
<th>BILASTINE</th>
<th>AZELASTINE</th>
<th>LEVOCABASTINE</th>
<th>OLOPATADINE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5 mg/ml = 0.05 %</td>
<td>55.80</td>
<td>45.67</td>
<td>53.59</td>
<td>49.17</td>
</tr>
<tr>
<td>0.5 mg/ml = 0.05 %</td>
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<tr>
<td>0.5 mg/ml = 0.05 %</td>
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<tr>
<td>1 mg/ml = 0.1 %</td>
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</tbody>
</table>

The activity of Bilastine is similar to that of Levocabastine, which is never better than Bilastine. There is no significant difference between equal concentrations of the two products. Nevertheless, both Bilastine and Levocabastine show higher potency than Olopatadine and Azelastine.
In vitro Toxicology Studies

• Determination of Bilastine Growth Inhibition Concentration on the most representative cell type of human cornea (epithelium and fibroblast).

  Human Corneal Epithelium: Bilastine showed an Inhibitory Growth Concentration 50 (IC50) upper 250 micromolar.
  Human Corneal Fibroblast: Bilastine showed an Inhibitory Growth Concentration 50 (IC50) upper 500 micromolar.

• BALB/C 3T3 cell phototoxicity Assay
  A not calculable PIF or a MPE <0.1 predicts “no phototoxicity”. On the basis of the results obtained, Bilastine should be classified as “non phototoxic”.

Ex vivo Toxicology Studies

• Bovine corneal opacity and permeability Assay (BCOP), OECD 437
  Bilastine is not considered to be corrosive to the eye and according to the INVITTOX (UK) protocol no. 98, is most likely classified as non eye irritant. Finally, the cornea were fixed and histopathologically analyzed and Bilastine caused no morphological alterations in bovine corneal samples.

• Irritation test in Hen’s egg chorioallantoic membrane (HET-CAM)
  With the HET-CAM (Hen’s Egg Test – Chorioallantoic Membrane) test an irritant or corrosive potential of the test substance can be determined by the detection of damages in blood vessels under the chorioallantoic membrane of fertilized and incubated chicken eggs.

  Bilastine can be stated not to possess any irritating potential.
**In vivo Toxicology Studies**

- **Primary eye irritation in rabbits**
  The primary eye irritation potential of Bilastine was investigated according to OECD test guideline no. 405. The test item was applied by instillation of 0.054 g, corresponding to 0.1 mL (according to preliminary test formulation) into the right eye of each of three young adult New Zealand White rabbits. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, after test item instillation.

  **Bilastine is considered to be “non irritating” to the rabbit eye.**

- **4-week eye tolerance toxicity study in rabbits**
  The purpose of this study is to assess tolerability to Bilastine eye drop when administered by topical route to the conjunctival sac of rabbit eyes twice daily for 28 consecutive days.

  Based on the results obtained in the study, topical administration of Bilastine to the conjunctival sac of rabbit eye for 28 consecutive days, twice a day at approximately 3-hour intervals did not cause relevant ocular alterations; thus, demonstrating **ocular tolerability.**
**Toxicological Studies II**

- **Corneal Anesthesia in Guinea Pig**

  The purpose of this study was to evaluate the local anesthetic activity of Bilastine in guinea pig by measuring corneal sensitivity after topical ocular administration.

  Ocular sensitivity in the guinea pig decreased slightly after topical application of Bilastine although differences were statistically significant with respect to the Control group only at one post-administration time (25 minutes).

  Effect on this particular point of time together with lack of effect at other times observed lead to conclude that these data have no particular relevance on overall results.
• At minimal dose with maximal activity or usual concentration, Bilastine shows higher potency than Olopatadine or Azelastine.

• Levocabastine has never been better than Bilastine.
**CONCLUSIONS (II)**

**TOXICOLOGICAL DEVELOPMENT (under GLPs)**

- **Phototoxicity Assay on Fibroblast Culture**
  Bilastine is classified as non phototoxic

- **Bovine corneal Opacity and Permeability (BCOP)**
  Bilastine is not irritant to eyes (Invittox Protocol 98 UK)

- **Irritant test. Chorioallantonic membrane. Hen's egg**
  Bilastine can be stated not to possess any irritating potential.

- **Primary eye irritation in rabbits**
- **4-week eye tolerance in rabbits**
  Bilastine is “non irritating” and demonstrates ocular tolerability.

- **Corneal Anesthesia**
  No revelant results on anesthesia
All these results together present Bilastine as a product with higher potency than other antihistamines (Azelastine and Olopatadine) and with a clear security profile in preclinical toxicology.

Strong collaboration help getting to the clinical phases of drug development.
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