Development of Epicorneal Conjunctival Membrane on Eyes During a One Year-Rabbit Intraocular Toxicity Study

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KEY WORDS
Pseudopterygium, rabbit, eye, conjunctiva.

SUMMARY
Eight of the 60 Dutch rabbits in a one-year intraocular toxicity study developed clinical ophthalmological changes characterized by a progressive extension of the bulbar conjunctiva to cover part or all the cornea. The progression varied among the animals and there was no concordance with the contralateral eye in the same animal. Histopathological examination showed a fold with its origin in the conjunctival sacs. This fold was formed by a connective tissue core lined by a conjunctival epithelium. Secondary inflammatory changes were only seen in the most severe cases. Minor histopathological changes were detected in about 24 of the 52 rabbits without any clinical changes. This ocular change was clearly unrelated to treatment since it was seen in both treated and untreated eyes. This spontaneous proliferation had some similarities with pterygium described in humans such as location in the interpalpebral fissure and extension of conjunctival tissue. There were, however, marked differences in the clinical appearance and histopathological structure as the lesion involves the superficial part of the cornea in human pterygium but is localised between the cornea and the eyelid in the rabbit. A few similar cases are described in the literature as pseudopterygium, hyperplasia, precorneal membranous occlusion or epicorneal conjunctival membrane. The aetiology and incidence in different strains of rabbits and pathophysiology of this spontaneous change are unknown.

INTRODUCTION
The development of uncommon pathological changes during the course of a regulatory toxicity study is always difficult to interpret, particularly in determining whether the “test article” was the cause of the changes or if it is a truly spontaneous change.

In the course of a one-year intra-ocular toxicity study in the Dutch Belted rabbit, a progressive extension of the bulbar conjunctiva covering part or all of the cornea was observed clinically as an incidental finding in eight out of sixty animals. Additional cases were detected at the histopathological examination in 24 of the 52 animals with no clinical changes.

Similar changes, described under several terms, have already been reported as an occasional finding, particularly in the dwarf rabbit. It is not known to what extent this change can be compared to the human pathological condition of pterygium.

MATERIAL AND METHODS
Thirty male and thirty female 3.5 to 5.5 month-old SPF Dutch Belted (pigmented) rabbits were obtained from Harlan UK Ltd. They were housed in an air-conditioned building between 16 and 22°C, with a relative humidity of at least 45% and at least 12 air changes per hour. The lighting cycle was of 12 hours light (artificial) and 12 hours dark per day, with an intensity ≥400 lux at a distance of one meter of the ground. Animals were housed individually in stainless steel cages with grids. Feces were collected in trays lined with paper which were cleaned every day. Each rabbit was fed with 150 g per day of a complete sterilised pelleted rabbit diet (UAR, Epinays/Orge, France). Softened filtered mains drinking water was given ad libitum via an automatic system. Diet and water were analysed for the absence of chemical and bacteriological contaminants.

A clinical examination for ill health was performed on arrival and the animals were observed...
during a two-week acclimatisation period, then allo-
cated to three treatment groups. Treatment was per-
formed once daily in the morning seven days per 
week for 52 weeks. Each administration consisted of 
a topical administration of a new ophthalmic prepa-
ration intended for human use by instillation of one 
drop (about 30µl) into the conjunctival sac of the 
eye. There were three groups in the study. Group 1 
animals (controls) received nothing in the right eye 
and the vehicle in the left eye, group 2 and 3 ani-
imals received the vehicle in the left eye while the 
right eye received the test substance at two different 
concentrations. Animals were observed during the 
acclimatisation period, then twice daily at the begin-
ing and at the end of the working day to detect any 
clinical signs or reaction to treatment. A general ex-
amination of the eyes was performed weekly. A com-
plete ophthalmological examination with a slit lamp 
and indirect ophthalmoscopy was performed at 
weeks 12 or 13, 25 or 26 and at termination (week 
52). Individual body weight and food consumption 
were measured weekly.

At the end of the study, animals were sacrificed 
with a lethal intravenous injection of pentobarbital 
then exsanguinated by section of the femoral ves-
sels. After the death both eyelids were stitched to-
gether. The whole globe with both eyelids were sam-
ped together, fixed in Davidson's fixative and 
processed into paraffin. Histopathological examina-
tion was performed on a sagittal section of the eyes 
including both eyelids. Slides were stained with 
haematoxylin and eosin.

RESULTS

Clinical observations

Occasional clinical signs including soft faeces, 
watering of the eyes and coloured urine were seen 
ocasionally during the treatment period. None of 
these were considered to be related to treatment.

There were no signs of local irritation due to either 
the test article or the vehicle.

Ophthalmological findings

Ophthalmological examination revealed changes 
described as a progressive translucent, well vascu-
larised, extension of the bulbar conjunctiva covering 
part or all of the cornea in eight animals (Fig. 1 & 
2). In the early stage of the onset, the lesion was 
more obvious in the upper part of the eye (Fig. 1), 
but was progressively broadly symmetrical around 
the periphery of the eye giving a ring-like partial oc-
cclusion. This was bilateral in four animals and uni-
lateral in the other four animals. In total 12 eyes 
were involved: two untreated eyes, seven eyes treat-
ed with the vehicle and three treated with the test 
article. The time of the first appearance, the rapidity 
of growth and the severity varied between individu-
als and even between eyes in the same animal 
(Table 1). In two cases, the first observation was 
made during the pretest examination. No new cases 
were seen after week 17 and no regression was ob-
served in any of the affected animals. In two ani-
mals, more than three quarters of the cornea was 
covered after 13 weeks (Fig. 2). Although in the

<table>
<thead>
<tr>
<th>Animal Number</th>
<th>Sex</th>
<th>Side Right/Left</th>
<th>First observation (week)</th>
<th>End of evolution (week)</th>
<th>Extent on the cornea at the end of treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F</td>
<td>R* L</td>
<td>7</td>
<td>45</td>
<td>3/4 of the surface 1/4 to 1/2</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>R L</td>
<td>17</td>
<td>17</td>
<td>&lt;1/4</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>R L</td>
<td>Pretest</td>
<td>Pretest</td>
<td>&lt;1/4</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>R* L</td>
<td>7</td>
<td>24</td>
<td>1/4 to 1/2</td>
</tr>
<tr>
<td>5</td>
<td>M</td>
<td>L</td>
<td>13</td>
<td>37</td>
<td>1/4</td>
</tr>
<tr>
<td>6</td>
<td>F</td>
<td>R L</td>
<td>Pretest</td>
<td>42</td>
<td>1/2</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>L</td>
<td>9</td>
<td>36</td>
<td>1/4</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>L</td>
<td>7</td>
<td>20</td>
<td>3/4</td>
</tr>
</tbody>
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R: Right, L: Left, *Photo taken.
most extended cases these changes obviously im-
paired the vision, no tests were performed to evalu-
ate this impairment. The animals did not show any
differences in body weight or food consumption. At
necropsy, it was possible to introduce a small spatu-
la to a depth of a few millimeters between the cornea
and this extension of the bulbar conjunctiva.

**Histopathological findings**

Histopathological examination of the eyes revealed
a connective tissue core with its origin in the con-
junctival sac. The core was lined by a normal con-
junctival epithelium. The extent of the fold seen his-
tologically varied greatly, but in general correlated
with the clinical observations. Minor folds that had
not been detected clinically were, also, seen histolog-
ically in 24 of the 52 rabbits without clinical changes.
These were bilateral in nine animals and unilateral in
fifteen. The folds were not generally associated with
any primary inflammation or other abnormalities. In
four eyes, focal adhesions were seen between the fold
and the cornea (Fig. 3), secondary squamous meta-
plasia of the cornea and/or the conjunctiva were ob-
served in four eyes and in the most severely affected
animals (two eyes), fragments of detached hair be-
tween the fold and the cornea (Fig. 4) were observed
given rise in one animal to a secondary keratitis.

**DISCUSSION**

There were no changes that were considered to be
associated with either the test article or the vehicle.
Unilateral or bilateral watering in the eyes was seen
occasionally in control and treated animals. This oc-
curred both in the treated and/or the vehicle control
eyes and was therefore considered not to be treat-
ment-related. Such a finding is commonly seen in
rabbit facilities and is considered to be associated
with presence of foreign bodies in the eyes (eg. hair).

Based on these clinical, ophthalmological and his-
tological findings, the diagnosis of pseudopterygium
was made in a first instance, taking in account the
similarities and dissimilarities with what is de-
scribed in human pathology as pterygium. As simi-
lar findings were seen at the pretest examination in
two animals and in eyes given either one of the vehi-
cles or the test substance and in untreated eyes, a
treatment-related effect could be eliminated, sug-
gesting a spontaneous pathology.

Similar changes have been described in the litera-
ture, particularly in the pygmy or dwarf rabbit1,2,
however, no references to similar lesions were found
in other species. Various other terms, such as hyper-
plasia, epicorneal conjunctival syndrome1,6,8, or pre-
corneal occlusion2 have been used to describe the
clinical aspect of these spontaneous changes seem-
ingly similar to our observations. The clinical ap-

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**Figure 1:** Epicorneal conjunctival membrane covering part of the cornea.

**Figure 2:** Epicorneal conjunctival membrane covering the whole cornea.

**Figure 3:** Adhesions between the fold (epicorneal conjunctival membrane) and cornea with minimal squamous metaplasia.

**Figure 4:** Extensive fold (epicorneal conjunctival membrane) with detached hair and corneal squamous metaplasia.
Pearce described unilateral or bilateral changes in the clinical changes. Other reports from the literature seen bilaterally in four of the eight animals showing rabbit.

A body which is a fibrovascular mass demarcated to an elevated white mass adherent to the globe and subepithelial corneal opacity, a head corresponding in the human pterygium: a cap which is a grey-white but was not seen in these rabbits.

Condition called pterygium. Some similarities with the human pathological val growth in the interpupillary fissure, which suggested some similarities with the human pathological condition called pterygium.

Both entities, in the rabbit and in man, are located in the interpupillary fissure and correspond to an extension of conjunctival tissue onto the cornea. In man, pterygium has a typical triangular shape and tends to be located nasally, though it can occur temporally. In the rabbit, changes involved the whole eye and had an annular shape. In human cases of pterygium, both eyes are involved in about one third of patients, whereas in our study changes were seen bilaterally in four of the eight animals showing clinical changes. Other reports from the literature described unilateral or bilateral changes in the rabbit.

Three regions of the lesion are typically described in the human pterygium: a cap which is a grey-white subepithelial corneal opacity, a head corresponding to an elevated white mass adherent to the globe and a body which is a fibrovascular mass demarcated from normal conjunctiva by sharp folds. In our study, the membrane structure was homogeneous. Tear pooling can be seen in the human pterygium, but was not seen in these rabbits.

In both man and rabbits these ocular structures are markedly vascularised and have a tendency to grow, although in both species these may become quiescent. The relationship of these abnormal ocular structures with the cornea was also different. In advanced cases, pterygium encroaches onto the cornea, but no primary adhesions with the cornea were apparent in the rabbit, since it was possible to slide a thin spatula between the cornea and the membrane.

Histological examination showed also marked differences between human pterygium and what was observed in our rabbits. In the human pterygium, changes clearly involved the cornea and consisted of a fibroelastic tissue which separates the basal corneal epithelial layer from the Bowman’s layer with altered orientation of the basal epithelial layer of the cornea and destruction of the Bowman’s layer. In the rabbit, however, the connective tissue core was not seen under the epithelial layer of the cornea but between the eyelid and the corneal surface without primary damage to the corneal structures. Although some adhesions were seen histologically in animals showing the most advanced changes, these were not considered to be primary changes, but most likely a consequence of irritation either due to rubbing or to the presence of hair follicles. In the human pterygium, the subepithelial layers of the substantia propria of the cornea show hyalination with presence of cosinophilic granular material. In addition, numerous thickened and ab-normal elastic fibers are observed. No signs of degenerating fibers were observed in our rabbits. The connective tissue core only appeared to be a fold arising from the conjunctival sacs. In the human pterygium, occasional cases of squamous cell metaplasia, acanthosis and dyskeratosis were observed in the epithelium of the pterygium.

The aetiology of these changes is not clearly defined in either species. Several epidemiological studies showed that the human pterygium occurs with a greater incidence in the equatorial regions of the world. Environmental factors, particularly increased exposure to U.V. or chronic irritation due to irritants (eg. dust particles) might be involved. One case of pterygium associated with a formaldehyde-containing nail hardener has been reported in man. Similarly, the aetiology of these changes in the rabbit is unknown as is their incidence in different strains. The involvement of a congenital or acquired abnormal differentiation of the epithelium and connective tissue at the limbus has been proposed as a contributing factor. The similar changes described in the pygmy or dwarf rabbit and the incidence at a low severity during the pretest examination in our study suggest a congenital predisposition. The reported treatment in both man and the rabbit consisted of surgical removal of the membranes from the globe followed by adjunctive therapies including chemotherapy with antiproliferative agents such as mitomycin C and radiotherapy in human beings and antibiotic ointments in the rabbit. In both the human and the rabbit, however, secondary growth of the membrane following surgery of pterygium is not uncommon.

As ocular changes seen in these rabbits have some similarities with the human pterygium (fibrovascular tissue extending from the conjunctiva in the interpupillary fissure), but also many differences regarding the clinical and histological appearance, the term pterygium was considered not to be appropriate to describe changes seen in the rabbit. The term pseudopterygium would be appropriate to take into account the clinical similarities and dissimilarities. Unfortunately, in veterinary or human medicine, pseudopterygium refers to a well-known entity that consists of a “conjunctival scar attached to the cornea and superficially resembling a true pterygium, but usually not firmly adherent to the underlying tissues”. In addition, the aetiology of pseudopterygium is known since it is a sequel of corneal trauma. In the absence of a well-defined and non-ambiguous term to define the changes in our study, a descriptive term, such as “precorneal occlusion” or “epicorneal conjunctival membrane”, terms which were already used in the literature seemed to be the most appropriate.
ACKNOWLEDGEMENTS

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REFERENCES