CONGENITAL ANIRIDIA KERATOPATHY TREATMENT

MANEJO TERAPÉUTICO DE LA QUERATOPATÍA ASOCIADA A ANIRIDIA CONGÉNITA

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ABSTRACT

Objective: To attempt to review the aniridia keratopathy pathogenesis and establish a treatment protocol according to the severity of the symptoms.

Methods: Personal experience in aniridic keratopathy management and a bibliography review.

Results: The ocular manifestations of this anomaly include defects of the cornea, glaucoma, lens subluxation, cataracts, hypoplasia of the iris, fovea and optic nerve, amblyopia and nystagmus. The keratopathy occurs in a 20% of patients with aniridia. The correct PAX6 expression is necessary for normal corneal development, limbal stem cell activity and correct corneal epithelial cell migration and adhesion.

Conclusions: The management of ocular surface diseases due to limbal stem cell deficiency in aniridia is complex but has changed in recent years, as an understanding of the limbal stem cells and their microenvironment has modified the therapeutic approach. The use of autologous serum eye drops, amniotic membrane transplantation, limbal transplantation or cultivated limbal cell transplantation have all been reported as a treatment for several...

**Key words:** Aniridia, limbal deficiency, autologous serum, ocular surface, dry eye, corneal epithelium.

**INTRODUCTION**

Aniridia is an infrequent disease (1:65,000-95,000) caused by a bilateral alteration in the eye’s development. It has no gender or racial preference. It is a well-documented genetic anomaly which may appear sporadically or within families, exhibiting a dominant autosomic inheritance pattern with variable expression amongst the members of a family. The gene which accounts for the disease (PAX6) is located in the short arm of chromosome 11 (1) and finds broad expression in the development of various eye structures including the cornea, lens, cameral angle, ciliar body and all retina layers. Therefore, aniridia is a global eye disorder in which iridian hypoplasia (which gives its name to the disease) is only the most evident clinical sign. The alterations in the anterior segment include keratopathy due to limbus dysfunction, dry eye, glaucoma, cataract, lens subluxation and a number of abnormalities in the cameral angle. The posterior segment alterations include macular and optic nerve hypoplasia with a frequent association of strabismus and nystagmus, which worsen the visual prognosis since an early age (2) (fig. 1).

The first description of the disease was made by Barratta in 1818, who gave it the name of irideremia in relation to the most apparent clinical sign. In the early twentieth century the current denomination began to be used.

In humans, PAX6 gene mutations were found not only in patients with aniridia, but also in some case of Peter’s anomaly. Said mutations can be intragenic or deletions of the 11p chromosome. No correlation was found between the mutation site of the PAX6 gene and variations in the expression of the aniridia phenotype expression. In fact, a broad range of phenotypes can occur as a consequence of the same mutation.

Among the most important extraocular alterations we find the development in sporadic cases of Wilms tumor, frequently as part of the WAGR syndrome (Wilms tumor, aniridia, genital-urinary abnormalities and mental retard). The appearance of these extraocular alterations in aniridia is more common in sporadic cases. When aniridia is diagnosed in a child whose family does not have antecedents of the disease, it is important to establish via chromosome analysis whether or not the deletion extends to the domain of the gene which predisposes to Wilms tumor in order to establish a monitoring regime for the period of risk of expression of this tumor, which is usually up to age 8.

Although the phenotype may vary considerably, even among members of the same family, the visual defect appears in the first decade of life with visual acuity values ranging between 10 and 20% due to foveal hypoplasia combined with the possible existence of congenital cataract, nystagmus and amblyopia (3). In contrast with the stability of retinal alteration, the progressive deterioration of the eye-sight is mainly due to the development of glaucoma (50-75%), cataract and keratopathy.

Keratopathy associated to aniridia (KAA) affects 20% of patients, although alterations in the eye sur-

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**Fig. 1:** Keratopathy associated to aniridia. The illustration shows the superficial neovascularization and subepithelial fibrosis.
GENETIC BASES AND PATHOGENIC MECHANISMS IN KERATOPATHY ASSOCIATED TO ANIRIDIA

PAX6 is the gene responsible for aniridia. It is found in the 11p13 chromosome segment (4-6). The majority of recent studies on the genetic bases of hereditary aniridia are based on animals, particularly mice. The gene PAX6 homozygotic alteration models give rise to mice with complete absence of eyes and nasal cavities which die soon after birth. The heterozygotic defect of PAX6 produces the Sey (Small eye) mouse model with microphthalmus and ocular alterations similar to those seen in humans with congenital aniridia, therefore regarded as a good experimental model for the disease.

PAX6 is contained in a region of DNA considered to be of an extraordinary phylogenetic importance because it has been conserved in numerous species of vertebrates and invertebrates throughout the evolutionary process (7). This gene is considered to be an important regulator in ocular morphogenesis, controlling cellular proliferation, differentiation and apoptosis processes in normal eye development. The normal expression of PAX6 is deemed essential for the development of ocular tissue such as the corneal epithelium, iris, ciliary body, lens and retina (8-10).

The regulatory role of PAX6 continues in adult life and occurs at several levels.

Regulation of the proliferation and differentiation of the corneal epithelium

The absence of a selective marker for the limbus stem cells (LSC) hinders the study of the epithelial proliferative compartment in animal models. The results obtained with the p63 nuclear transcription factor, suggested as a possible marker for epithelial stem cells and for other, more differentiated cells such as the amplified transient cells, doesn’t seem to exhibit alterations in the PAX6 mutation models. In addition, conclusive results were not obtained with cell proliferation markers such as BrdU (11-13). The expression of CK-12 and CK-3 through the epithelial cells is considered to be a differentiation marker more specific for the cornea and besides it is regulated by PAX6. These are water insoluble cell cytoskeleton proteins which make up the intermediate filaments of the epithelial cells. Their main function is to stabilize the corneal surface. In aniridia experimental models we find a reduced expression of these cytokeratins, which translates in vacuolization and fragility of the corneal epithelium (14,15).

Regulation of cell adhesion

The corneal epithelial cells have numerous adhesion mechanisms, both intra- and inter-cellular as well as with the extra-cellular matrix. Said mechanisms include «tight-junctions», «gap-junctions», desmosomes and adhering unions. In addition, there is a large variety of adhesion molecules such as catenines, integrines, demogleine and desmocholine which make the corneal epithelium highly resistant to external attacks.

In experimental aniridia models we find a reduction in demogleine as well as beta- and alpha-catenine, the synthesis of which seems to be regulated.
by gene PAX6, which gives rise to spaces between epithelial cells (16). These findings, together with the cytokeratine CK-3 and CK-12 deficit, make the corneal surface very fragile. The clinical expression of this is the presence of recurring erosions or persistent epithelial defects (17).

**Regulation of extra-cellular matrix activity**

The extra cellular matrix gives the cornea its structural organization. In normal conditions, the extra cellular matrix is subject to a slow but continuous remodeling which gathers pace in the process of repairing corneal damage (18,19). There is a balance between the production of collagen by keratocytes and their degradation by a group of enzymes called metaloproteinases (MMP). These enzymes are produced by epithelial cells and fibroblasts, and their expression is influenced by genes, growth factor, tissue inhibitors of metaloproteinases and elements of injuries. In turn, said enzymes are involved in the activation of cytokines, in the rupture of adhesion molecules and the creation of biologically active fragments (20,21). Three types of metaloproteinases are known: MMP-1 or collagenase type 1, MMP-3 or stromelysine and gellatinase. All these are secreted as proenzymes and need zinc as a cofactor (22). MMP1 is produced mainly by keratocytes and degrades collagen type 1, 2 and 3. MMP-3 exhibits an intense proteolytic activity vis-à-vis caseine, fibronectine and proteoglycans. In turn, gellatinase is more selective for gellatin and collagen type 4, 5 and 7. Two types of gellatinases are differentiated: MMP-2 is more active in the presence of collagen type 4, whereas MMP-9 is more active in the presence of collagen type 5. The regulation of Gel-B or MMP-9 depends on PAX6. In PAX mutation animal models with Gel-B deficiency we find an accumulation of fibrine and infiltration by inflammatory cells in relation to an increase in the levels of IL-1 (23). The accumulation of fibrine alters the orderly arrangement of collagen fibres, producing loss of corneal transparency. On the other hand, cellular infiltration translates into an important neovascular proliferation stimuli.

Figure 3 shows the different actions of gene PAX6 on the eye surface and its clinical consequences.
helium, chronic inflammation, conjunctival hypere-
mia, changes in Bowman’s membrane and kerati-
ization. Symptoms include crying, pain, eyesight 
reduction, dry eye, photophobia and blepharos-
pasm, with the frequent addition of bacterial infe-
tions and the risk of eye perforation (27,28).

The instability of the ocular surface which is cau-
sed by the deficient regeneration of the corneal 
epithelium will determine, among other things, the 
appearance of a secondary dry eye and the loss of 
the corneal epithelium’s barrier function (fig. 4). 
This chronic aggression on the eye surface epithel-
ium will produce a reaction of the latter in the form 
of metaplastic transformation. Accordingly, squa-
mous metaplasia is a process utilized by humid 
epithelia to provisionally overcome attacks. This 
process involves both corneal and conjunctival ep-
thelial cells as well as the conjunctiva’s secreting 
cells (calliciform cells) (29). This is a pathological 
and reversible transition of the normal, non-kerati-
nized stratified epithelium to keratinized epithel-
ium.

Clinically, the keratinization process is easily 
recognizable via microscopy but squamous meta-
plasia which arises before keratinization can go 
unnoticed, above all in the initial stages. In these 
cases, impression citology provides an objective 
estimate of the degree of involvement of the ocular 
surface, allowing an improved therapeutic approach 
to the disease (30). In addition to assessing the 
degree of squamous metaplasia, citology will allow 
the identification of the presence of calliciform cells 
in the corneal epithelium, a characteristic conjunc-
tivalization sign and therefore signalling limbal 
insufficiency (fig. 5). Impression citology also facili-
tates the epithelial phenotype study via marking 
with monoclonal antibodies of selective citokeratines 
of each cellular lineage (31,32). Accordingly, the 
conjunctival epithelium cells present citokeratine 
CK19, whereas the corneal and limbal epithelial 
cells present citokeratines CK3 and CK12 (33). The 
cornea of patients with LSC deficiency present a 
reduction of citokeratines CK3 and CK12 and an 
increase of citokeratine CK19 (34). Furthermore, 
impression citology allows a study of the ocular 
surface after interventions such as limbus transplant 
and amniotic membrane transplant (35,36).

It is important to classify all the above signs and 
symptoms in order to plan the therapeutic handling 
of these patients. We have differentiated four levels 
or stages of development. A patient is considered to 
exhibit slight limbal insufficiency (phase 1) when 
he/she refers a maximum of two recurring ulcer or 
erosion events in the past 6 months, slight photop-
hobia and epiphora and also exhibit slight vascular 
pannus not exceeding 1 mm from the limbic arch 
and small disorders in the absorption of fluorescein. 
A patient is considered to exhibit moderate limbal 
insufficiency (phase 2) when the number of recur-
ing ulcer or erosion events equals or exceeds 3 in 
the past 6 months, exhibits permanent instability of 
the lacrimal film and vascular pannus (with or with-
out sub-epithelial fibrous tissue which involves at 
least the peripheral half of the cornea, with photop-
hobia, epiphora and red eye being the norm. The 
third phase of the disease is considered to be rea-
ched when a patient exhibits corneal vascularization 
involving the centre of the cornea as well as perma-

**Fig. 4:** Tincture with sodium fluorescein, showing conjunctivalization of the corneal epithelium.

**Fig. 5:** Impression citology. The presence of calliciform cells in corneal impression citology defines the instability of the lacrimal film and an epithelial defect.
nent clinical signs of corneal erosions and instability of the laterial layer. Photophobia, epiphora and red eye are the norm, together with the loss of vision due to involvement of the visual axis. Finally, in phase «0» or sub-clinical limbal insufficiency we include patients with etiological processes liable to limbal insufficiency who do not express associated clinical signs (fig. 6).

**TREATMENT**

One of the causes of progressive loss of vision and morbility in aniridia patients is keratopathy derived from the dysfunction of LSCs. The progress in recent years in the understanding of the mechanisms involved in cellular renewal of the cornea have allowed an adequate therapeutic approach of these patients in any phase of the disease (37,38). However, to date it hasn’t been possible to demonstrate in KAA patients the existence of a LSC deficiency due to the lack of specific markers allowing for their identification in histological studies (39,40), as the clinical findings in these patients coincide with those described in the limbal insufficiency syndrome.

Until about 3 years ago, the approach to KAA was based on supporting treatment with topical lubricants, therapeutic contact lenses or tharsorrhaphy and, when patients developed severe corneal opacity, lamellar or penetrating keratoplasty was adopted with very negative results due to recurrence of pre-surgical corneal alterations on the graft (27,41).

As with the rest of limbal insufficiency patients, treatment should aim at repopulating the sclerocorneal limbus of the LSC and/or to restore the micro-environment surrounding them in order to ensure their expansion and survival.

The therapeutic management of these patients will depend on the degree of involvement of the ocular surface. Thus, in patients exhibiting sub-clinical or slight limbal insufficiency, treating with artificial tears without preservatives could suffice. Although there are many tear drops in the market, we prefer to use sodium hyaluronate preparations free of preservatives. It is also important to apply with these patients a number of measures aimed at improving their symptoms, such as protection against sunlight by means of dark glasses, or a preference for humid environments. The corneal erosion events shall be treated just like in any other patients, by means of occlusion and topical antibiotics. In patients with slight keratopathy, we have tried treatment cycles with autologous serum and found a subjective improvement and a reduction in the number of corneal erosions (unpublished results).

In patients with moderate keratopathy, treatment with artificial tears is not enough. In these patients autologous serum or amniotic membrane transplant can be utilized. The former has proved to be a highly interesting therapeutic weapon for handing various pathologies involving the ocular surface (42,43). As for autologous serum, it involves many factors such as the epithelial growth factor, fibronectine, vitamina A, the fibroblast growth beta transforming factor, α2macroglobuline and neural factors such as the P substance, which act upon the proliferation, migration and differentiation of corneal epithelial cells (44,45). In this regard, autologous serum would have an effect similar to that produced by the amniotic membrane, contributing to improve the environment and facilitating the mechanisms involved in the cellular renewal and maintenance of the ocular surface epithelium (46). The amniotic membrane transplant (AMT) exhibits important clinical advantages as it improves considerably the environment of the extracellular matrix of the limbar epithelial cells (47). The therapeutic usefulness of the AM is derived from its mechanical and above all biological properties (48,49). This makes it very useful for patients with partial limbar insufficiency where the aggregation of multiple factors enhances the development and expansion of the surviving LSCs. AMT has been utilized for limbar insufficiencies associated to various etiologies (50,51) as well as in the treatment of aniridia-asso-
ciated keratopathy (52). However, medical treatment by means of AMT in these patients provides only temporary results, as we proved in aniridia patients (52) (fig. 7).

In patients with severe stage 3 keratopathy, the stabilization of the corneal surface involves supplying LSC by means of a limbus transplant (53,54). Aniridia being a bilateral disease excludes a self-transplant, which are substituted by allografts with tissue from healthy relatives having high HLA compatibility, or from cadavers (55). In these cases, the risk of rejection requires the application of a postop oral immunosuppressor treatment with potential side effects. The limbus transplant from relatives allows for greater HLA compatibility, thus partially reducing the rejection risk although it does not allow for obtaining grafts as large as those obtained when the limbal tissue comes from cadaver doners.

Homologous lamellar limbokeratoplasty has been performed in patients with bilateral aniridia with quite acceptable results (56). The combination of limbus transplant and AMT increases the benefit of each separately (35,57). In addition to the typical limbus transplant, at present we can supply LSCs grown artificially on amniotic membrane (58-60). The main advantage of this technique is that an important number of cells can be transplanted from a small limbus sample obtained from the same patient. This considerably reduces surgical trauma in the eye and the risk of rejection of the limbus allotransplant. Although good results have been described with these techniques for handling various ocular surface pathologies (61,62), in practice there are important issues to be resolved concerning the principles of this technique in the specific case of aniridia patients. On the one hand, there is no selective and specific method for identifying the LSCs and on the other, as mentioned above, aniridia seems to involve primarily an alteration of the environment surrounding the LSCs, with the quantitative alteration being secondary. Therefore, it is necessary to study the survival, mobility and adherence of epithelial cells after transplanting them in the eye of aniridia patients.

Figure 8 schematically illustrates the handling of the eye surface of KAA patients.

In addition to keratopathy, these patients frequently exhibit other anterior segment alterations such as cataract, glaucoma or corneal opacity requiring on some occasions surgical procedures such as trabeculectomy (63,64), corneal transplant (41) or phacoemulsification, with or without intraocular diaphragm implants (65,66). Particular attention should be given to treatment of glaucoma in these patients; whenever possible, preservative-free antiglaucomatous agents should be utilized to avoid the damages caused to the ocular surface by some preservatives included in pharmacological preparations routinely used for treating glaucoma.

Although it does not appear in all patients, KAA is a frequent cause of morbility. Therefore, an adequate therapeutic approach could be extremely useful to improve the quality of life of these patients. Finally, it can be said that aniridia patients are an important challenge for ophthalmologists due to the
frequent association of the macula, optic nerve, nystagmus and amblyopia disorders to the above-mentioned anterior segment disorders, which considerably reduce their visual capacity (67). On the other hand, photophobia can be highly invalidating, to the extreme that many patients require the iris prostheses (68,69). Lastly, we must not forget that in many cases aniridia is associated to other systemic disorders which also require our full attention (70,71).

REFERENCES

Aniridia keratopathy treatment


