INTRODUCTION

The term «Lamellar Keratoplasty» (LK) refers to those surgical techniques consisting of the replacement of pathological corneal tissue with healthy corneal tissue, leaving the unaltered corneal tissue from the receptor in position. In deep anterior lamellar keratoplasty (DALK) it is the anterior corneal tissue what is exchanged leaving the Descemet membrane (DM) – endothelium complex from the receptor intact1. Until recently, penetrating keratoplasty (PK), where the whole of the corneal thickness is replaced by a donor button, has been the surgical method of choice.

DALK offers a series of advantages against PK2; in the first place, loss of endothelial cells after DALK tends to be smaller than after PK. Another advantage is the decrease in the risk of endothelial rejection, since

TECHNIQUE

Development of an experimental animal model for deep anterior lamellar keratoplasty

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PURPOSE: The aim for the study was the development of an animal experimental model for deep anterior lamellar keratoplasty (DALK).

METHOD: Lohman Brown chickens were used as animal model. The study was divided into two separate phases: the first one comprised carrying out the surgery on the enucleated eyes donated by a local butchery for surgical training and selecting the technique the better adapts to the model. Several techniques for the dissection of the predescemetic plane were carried out, dissection with balanced solution, air, viscoelastic and nylon-silk 6/0 thread. On the second phase, for the study of the clinical evolution of the surgery and the healing mechanisms involved, surgery was carried out on one single eye of ten chickens using the modified Ferrara spatula dissection with 6/0 nylon-silk thread. Clinical follow up through surgical microscope was then completed up to 6 months, when the animals were sacrificed. For the histological study hematoxylin -eosin staining of the fixed corneas was done at 6 months.

RESULTS: The material was appropriately adapted and the dissection technique with nylon-silk thread and modified Ferrara spatula was selected for keratoplasty. In the second phase, surgery was successfully carried out on 7 chickens. As postoperative complications neovascularization in 4 eyes and anterior sinequiae in 3 eyes were found. Clinical follow up showed a moderate corneal swelling that decreased over time. Histological analysis showed fibroblastic reaction in the interface of both corneas.

CONCLUSION: An experimental animal model for DALK was developed in chicken. Histologically a fibrotic reaction in the interface exists and clinically there is a moderate degree of haze in the corneal center.

KEYWORDS: Anterior lamellar keratoplasty, nylon-silk 6/0 suture, corneal wound healing, chicken.

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receptor's endothelium is maintained and, even though epithelial or stromal rejection could occur, these can be more easily managed and offer a better forecast. In the third place, the lamellar procedure offers the patient a better and faster healing process since the integrity of the eye is preserved at all times, sutures may be removed sooner, medication reduced quicker, and the global health expense to keep a functional transplantation is lower than those for PK. Finally, the main indications for DALK are pathologies that affect the anterior portion of the cornea, and the fact that they are mainly found in young patients, makes the longer-lasting character of these transplants along with the lower dependency on the health service important.

For all that, the development of keratoplasty techniques allowing the preservation of part of the cornea from the receptor (in those cases where corneal pathology is not affecting the full thickness), aiming to avoid the complications previously described and make this technique a safer surgical procedure, is a challenge.

Currently, numerous DALK techniques have been described. These may be grouped depending on the dissection level into pre-descemetic, in which a small residual stromal bed is left in the receptor cornea, and descemetic, in which DM is exposed without residual stroma. Among these techniques it should be first highlighted the dissection with spatula or crescent under direct visualization after partial trepanation of the receptor cornea; not a very precise technique leading to a very irregular receptor bed. Later, Archila, Price and Chau described the intrastromal air injection to thicken and opacify the cornea in order to facilitate the progressive lamellar dissection and identification of DM membrane. After that, Sugita and Kondo proposed the balanced saline solution injection into deep stroma to swell collagen fibers (hydrodelamination) and therefore be capable of performing more easily the lamellar keratoplasty until all the stroma above DM is removed. Both techniques (air and balanced solution injection) have the same perforation rate (20-32%).

Melles et al proposed the Rotterdam technique in which dissection is carried out with specially designed dissectors, guided to the pre-desemetic plane by a series of corneal signs, after filling the anterior chamber with air. Anwar developed the «big bubble technique», in which after a partial trepanation of 60-80% corneal thickness an air bubble is introduced into deep stroma working as a guide for the partial dissection up to that pre-desemetic level. The disadvantage of this technique is the difficulty for introducing the air at the right place so that the «big bubble» is formed.

A more recent technique is that in which the Ferrara spatula, a modified version from that for manual implantation of intrastromal ring segments, is used for the dissection of the pre-desemetic plane and a 6/0 nylon-silk thread. Finally, several groups have assessed the use of laser for creating the pre-desemetic plane dissection. Both the Ferrara and laser techniques create a very precise ablation/dissection plane, being the main problems the high cost, the unavailability in every center and the difficulty in adapting the programs to these procedures.

Election of one technique or the other is generally made depending on the corneal pathology and the surgeon's personal experience with each of the techniques; One of the main drawbacks for the implantation of the DALK in clinical practice is the difficulty of the technique, the longer duration of the surgery with respect to PK, the existence of an interface between the donor and receptor corneas that compromises corneal transparency, the induction of irregular astigmatism and the lack of good models for the learning curve before getting into practice on human eyes.

The purpose of this work is developing an experimental animal model for DALK with the aim of validating its use for surgical practice prior to application on humans, secondly to aid in improving the knowledge on the different techniques and the healing mechanisms implicated, and finally to allow trying modifications for simplifying this technique and improving results thanks to this model.

**METHOD**

**Animals**

In order to get some practice with the technique and apply it correctly on chicken eyes, enucleated chicken eyes donated by a local butcher were used and, for the assessment of the technique, ten right eyes from ten living chicken were intervened.

For practicing the technique the eyes were fixated onto a fixation stand and different techniques for the dissection of predesemetic plane were tried out: dissection with air, balanced solution, viscoelastic, «big bubble» technique and nylon thread. For the study of the technique in evolution ten Lohman Brown chicken were used following the tenets of ARVO for the use of animals for research purposes. All chicken were allowed to evolve up to 6 months.

**Surgical technique**

Once the technique for the dissection of the predesemetic plane was selected as a function of the group experience on the different technique, it was applied on living chicken. Selected technique was dissection with modified Ferrara and nylon-silk 6/0 thread. Surgical material used was selected attending to the anatomical characteristics of chicken (lower corneal thickness and diameter as well as the presence of nictitant membrane) and provided by Ferrara Rings Co. (Valladolid, Spain).
Animals were anaesthetized with intramuscular 37.5 mg/kg Ketamine (Ketolar, Parke-Davis S.A, Barcelona, Spain) and 5mg/kg xylacine (Rompun, Bayer AG, Leverkusen, Germany), and topical anaesthesia instilled (0.5% tetracaine clorhydrate, and 1mg oxybuprocaine) (Anestésico doble colircusí, Alcon Cusi, Barcelona, Spain).

After topical anaesthesia a pediatric Barraquer blepharostat was held in place in order to keep the nictitant membrane fixed. In the first place, viscoelatic material was introduced into the anterior chamber through paracentesis using a 30 gauge rome cannula (Celoftal®) in order to have a more stable anterior chamber. Visual axis was then marked using as a reference the reflex of the microscope light onto the cornea. In order to do the tunnel marking, simultaneous tattooing of three concentric rings of 5, 5 and 7 mm was carried out using the Ferrara marker stained with gentian violet.

Prior to corneal pachymetry (Corneo-Gage Plus; Sonogage Inc, Cleveland Ohio), a radial incision was performed between the 5 and 7 mm circles calibrating the diamond blade to 90% of corneal thickness (Figure 1.A), in order to reach a predescemetic level. After that, a deep intraestromal pocket was dissected in the clockwise and anticlockwise direction using the Suarez spatula. This intraestromal pocket has the aim of serving as a guide for the introduction of the Ferrara spatula and dissect the intraestromal 360° tunnel. Later, the modified Ferrara spatula for chicken eyes (5 mm in diameter) was introduced, modified with a orifice at the distal side to thread a 6/0 nylon (Ethicon®-Johnson

Figure 1. Development of the surgical technique: A) Incision with the calibrated scalpel. B) Trepanation with the modified Ferrara spatula with a 6/0 nylon threaded. C) Dissection of the lamellae. D) Sutured cornea after the surgery.
the animals were sacrificed by cardiac sodium pento-
15, 21 days and 1, 3, 4, 5 and 6 months. At 6 months
surgical microscope for the first 7 days and then after
permeability, corneal haze and neovascularization) under
Follow up
Lab) every 8 hours for three days was administered.
ly proved by several studies carried out within our
tissue; with regards to the «big bubble» technique,
was carried out only on the right eye, according to cur-
from the stromal edge and, using a cellulose hemostat,
were carried out with great difficulty, obtaining
edema was observed along with DM
evolved favorably.
From a clinical perspective, during the first month
moderate degree edema was observed along with DM
striae, decreasing progressively with time, but not hav-
ing completely disappeared by the end of the study. In
the binding donor-receptor zone, a moderate stromal
haze was observed that increased in intensity and
decreased in area up until 3 months after the proce-
dure, when it started decreasing being stabilized at 6
months in a moderate degree. Anterior chamber reac-
tion was of moderate degree (Tyndall 2/4) and main-
tained throughout the first month, being progressively
reduced after that until practically disappear at 3
months (Figure 3). Anterior chamber was wide except
for those cases with complications.
From a histological point of view regarding the
cornea samples of 6 months of evolution stained with
Hematoxylin -Eosin and assessed using optical microscopy.
RESULTS
For practice and application of the technique on
chicken, several techniques were carried out: air dissec-
tion, balanced solution dissection, «big bubble» tech-
nique, viscoelastic dissection and nylon thread dissec-
tion. Dissections with air, balanced solution and vis-
coelastic were carried out with great difficulty, obtain-
ing very irregular receptor beds and residual stromal
tissue; with regards to the «big bubble» technique,
was also great technical difficulty with a high per-
foration rate, which made lamellar procedure impossi-
ble in many cases. Dissection with Ferrara spatula and 6/0 suture was
correctly carried out and with less difficulty, obtaining
lower perforation rates and more regular surfaces, and
for that reason it was considered the technique of
choice for DALK in chicken. Surgical interventions on
living chicken were successfully carried out on 7 eyes,
having intraoperative complications in 3 eyes; these
complications were perforations, which made the
lamellar procedure more difficult, having to change the
procedure to PK. Plane depth was similar for most of
the intervened eyes except for those with complica-
tions. With regards to postoperative complications,
severe inflammatory reactions in conjunctiva over the
first days were observed in 6 chickens (60%), decreas-
ing with time without significance, limbal neovascular-
ization towards the suture points were observed in 4
chickens (40%). Anterior iris to cornea sinequiae was
observed in 3 chickens (30%). The remaining corneas
evolved favorably.
From a clinical perspective, during the first month
a moderate degree striae was observed along with DM
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for those cases with complications.
From a histological point of view regarding the
cornea samples of 6 months of evolution stained with
Hematoxylin -Eosin the following must be highlight-
ed: at the incision zone a slight epithelial hyperplasia with fusion of both corneas (Figure 2) and loss of owman’s membrane at that level was observed; at stromal level, a great hypercellularity mainly located close to the donor-receptor cornea binding area was observed. Inflammatory cells were not observed. At the interface, a fusion of both corneas with the presence of fibroblast/myoblast morphology cells was observed. The number of endothelial cells compared with healthy corneas is similar.

**DISCUSSION**

The main aim of corneal transplantation is providing with corneal transparency and minimal astigmatism in order to achieve good vision for as long as possible.

PK has been the technique of choice until recently. However, numerous complications make it an aggressive technique to carry out. For that reason, development of techniques that allow keeping part of the cornea to make it safer and decrease the complications rate is a challenge.

We believe it is important to introduce this type of surgery in every Cornea Section from every hospital. Nevertheless, many limitations make this technique not to be extended to clinical practice due to its technical difficulty, lack of homogeneity in the predescemetic plane dissection creation, the limited visual results obtained in the first surgeries, etc. In the present work the chicken model was developed as animal model for DALK with nylon thread for the realization of the predescemetic plane. This model allows the reproduction of the original surgical technique described previously, with the same learning curve and reproducibility. It offers an ethically acceptable, cheap and anatomically similar to human, tool, being very useful for clinical practice, better knowledge of the technique and to try improvements with the aim of simplifying it and obtain better results.

Due to the use of living animals in evolution, knowledge on the mechanisms implicated in the corneal wound healing process could be improved using histological tools. Ethical limitations make impossible to carry out these studies in humans, and it is therefore necessary to develop an experimental animal model that allows the reproduction of the situation, modifying variables, and where response may be assessed. The rabbit has been used as experimental model, but differences exist compared to human in anatomy, biomechanical properties, as well as in the healing process, which could compromise the results obtained. Pig eyes have also been commonly used for surgical practice, but the difficulty in manipulating the living animal, along with its high cost and the anatomical differences with human corneas also make it difficult to use them as a model for evolution studies. For all that we propose the chicken model as experimental model for the development of the learning curve for the DALK and the study of the surgical technique. The chicken is ethically acceptable, cheap, easy to handle, and present similar anatomy and corneal response compared to humans. Chicken has been validated as experimental animal model through several studies within our group, corneal wound healing studies after several surgical techniques such as: photorefractive keratectomy (PRK), laser in situ keratomileusis (LASIK) and intrastromal ring segments.

The technique used for carrying out the DALK, the nylon thread for dissection at predescemetic level, has been recently described. This technique uses the instruments and basis of Ferrara’s intrastromal ring segment implantation surgery; The use of chicken model
for intracorneal ring segments is supported by several studies within our group, and therefore experience with the technique is considerable, minimizing possible complications coming from the first dissections at pre-descemetic level with nylon thread. The dissection performed with this technique offers a series of advantages: corneal bed created is homogenous and in one plane only, and secondly the fact that the scalpel may be pre-calibrated for the main incision as a function of the pachymetry in that location, allowing to obtain a predetermined plane quite accurately and offers the possibility of performing dissections at different levels and not just predescemetic. Another advantage derived in our group by the knowledge of the ring implantation technique, is the lower difficulty found with respect to other techniques for the dissection of the predescemetic plane, fact that may be shared with other corneal surgeons experienced in the manual implantation of intracorneal ring segments; and finally the possibility of applying the instruments of intracorneal ring implantation surgery for DALK, minimizing the expenses derived from the study. Despite the advantages regarding this technique within our group, the application of the chicken model for DALK when other dissection techniques are used is not ruled out.

With regards to the complications the 30% perforation rate observed falls within the perforation rates described in the literature, and the remaining complications (severe conjunctival inflammation, anterior synequiae and neovascularization) could be explained by the technical difficulty of the process and increased by the differences existing between the human and chicken corneas, although chicken constitutes a good tool for learning prior to application in humans in spite of that. From the histological point of view, data obtained are an example of the normal corneal stroma healing process. At first a fibrotic reaction occurs with fibroblast activation in order to fill the loss of tissue, favor the apposition of the edges and recover then the normal corneal morphology. Later, in an attempt to recover functionality, this fibroblastic reaction should disappear in order to recover transparency, fact that was not yet shown at 6 months.

The number of endothelial cells compared to normal healthy corneas is similar, indicating less surgical stress compared with data after PK, fact that strengthens the efforts to develop the technique described. Except in complications inflammatory cells were not observed indicating good tolerance to transplant in the absence of immunosuppressing therapy. Nevertheless new studies assessing longer evolution periods should be carried out in order to understand better the histopathological processes involved.

REFERENCES