Unexpected outcome (positive or negative) including adverse drug reactions

Successful deep anterior lamellar keratoplasty following multiple failed limbal transplantations for chronic ocular burns

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Summary

A 9-year-old girl with severe unilateral ocular surface disease due to limbal stem cell deficiency following chemical injury underwent autologous cultivated limbal epithelial transplantation (CLET) twice in her right eye that failed to improve the condition of the ocular surface. On both occasions, limbal biopsies were obtained from the unaffected left eye. Since further attempts at CLET were not considered to be safe, deep anterior lamellar keratoplasty (DALK) was performed instead. DALK was complicated by postoperative Descemet's membrane detachment, which was successfully treated. Two years post-DALK the patient had a clear graft, stable ocular surface and visual acuity of 20/80. The mechanism by which CLET allows a patient to repopulate the ocular surface is as yet unknown. This case highlights that although clinically little had changed following two failed CLET surgeries, some subclinical improvement must have taken place for a DALK to remain successful after 2 years.

BACKGROUND

Corneal scarring is the fifth commonest cause of preventable blindness worldwide, and accounts for 20% of childhood blindness according to the WHO. Damage to corneal stem cells gives rise to a painfully blinding condition, which was once thought of as incurable. Little is still understood as to the mechanism by which one of the main treatments (cultivated limbal stem cells transplantation, CLET) works, although the results are very promising. This case challenges the currently understood dogma that the stem cell reserve is based only in the corneal limbus. It also challenges the way we currently evaluate such patients, and opens up the possibility of corneal transplantation in these patients which was once considered impossible.

INTRODUCTION

The corneal epithelium is thought to be renewed by the progeny of stem cells located in protected niches at the limbus.¹ However, severe ocular surface inflammation or trauma can damage these stem cells and cause corneal epithelial dysfunction, loss of corneal transparency and blindness. Limbal stem cell deficiency (LSCD) is predominately a clinical diagnosis, with the main features being poor epithelialisation of the corneal surface, recurrent erosions, chronic stromal inflammation, corneal neovascularization and conjunctivalisation of the cornea. Patients usually present with pain, poor vision and corneal opacity. Causes of limbal stem cell damage that may lead to LSCD include thermal or chemical injuries, pterygium, ocular pemphigoid, Stevens-Johnson syndrome and mechanical damage of the limbus from multiple surgical procedures. The existing paradigm of managing eyes with LSCD is limbal transplantation from a healthy donor eye.² In

addition, 15–38% of eyes undergoing limbal transplantation need a keratoplasty for visually significant corneal stromal scarring.³ Past experience of corneal surgeons and current scientific evidence suggests that the chances of keratoplasty being successful in eyes with LSCD are best if it is performed following successful limbal transplantation. This is because keratoplasties performed prior to or along with limbal transplantation almost always fail due to early recurrence of LSCD.⁴ In this report we describe the ocular surface reconstruction in a patient with severe unilateral chemical injury using a combination of limbal transplantation followed by deep anterior lamellar keratoplasty (DALK). The point of interest in this case is that the DALK remained successful despite being performed following two supposedly failed limbal transplantations.

Case report

A 9-year-old girl was referred to the LV Prasad Eye Institute (LVPEI), Hyderabad, India with a diagnosis of LSCD in the right eye (OD) in December 2007, following accidental lime paste injury 2 years before. She had undergone symblepharon release with amniotic membrane grafting in August 2005, which was then repeated in August 2007 in another centre. The reason for referral to LVPEI was for symptom relief, as she had an increasingly painful eye and visual rehabilitation due to her deteriorating vision. The best corrected visual acuity was hand motions in the right eye and 20/20 in the left eye. The digital intraocular pressure was normal. External examination revealed extensive symblepharon and 360° corneal conjunctivalisation with extensive pannus (figure 1A). She was diagnosed as total limbal stem cell deficiency clinically. The supportive signs were poor epithelialisation of the corneal surface with recurrent erosions, chronic

Figure 1 (A) This slit lamp photograph depicts extensive symblepharon formation superiorly at the time of presentation. (B) Slit lamp photograph demonstrating corneal scarring, vascularisation and total limbal stem cell deficiency. (C) Slit lamp photograph of the failed cultivated limbal epithelial transplantation (CLET) with a vascularised corneal surface. (D) This slit lamp photograph demonstrates conjunctivalisation between 1 and 2 O'clock at the site of limbal biopsy for harvesting the CLET. (E) This is a slit lamp photograph of the right eye 2 months following the deep anterior lamellar keratoplasty with a clear graft in place and healthy looking epithelial surface. (F) This is the slit lamp image of the patient's corneal transplant which remains clear centrally, surrounded by arrested peripheral conjunctivalisation 22 months after treatment.

stromal inflammation and conjunctivalisation of the cornea. Due to the risk of amblyopia, the patient was referred to the paediatric team. She was not recommended to undergo any patching treatment as she had good vision prior to the injury aged 7 (therefore, full visual pathway development was assumed).

Anterior segment structures were not visible although ultrasound B scan was within normal limits. An informed consent was obtained from the parents to undertake a two-stage procedure of limbal biopsy followed by ex vivo CLET under general anaesthesia. The goal of surgery would be first symptoms relief, providing the eye with a stable and healthy cornea. The secondary goal would be visual rehabilitation, with some patients requiring corneal transplantation to achieve optimal visual outcomes. Transplantation alone is much more likely to fail in these cases as a stable source of epithelial cells is required to cover the donor cornea. Postoperatively she received ciprofloxacin 0.3% eyedrops four times daily for 1 week and prednisolone acetate 1% eyedrops six times daily tapered over the next 8 weeks with artificial tears. Unfortunately, she presented 2 months later with a painful red eye and was found to have a corneal infiltrate in the right eye. Subsequent corneal scrapings revealed the organism to be *Streptococcus pneumoniae* sensitive to all antibiotics. Although the corneal ulcer was successfully treated, LSCD recurred in the right eye (figure 1B). Her parents were counselled to undergo further treatment and so she underwent repeat limbal biopsy in the left eye, followed by CLET in the right eye 11 months following the initial surgery. Despite a normal postoperative course and appropriate medication use the patient was left with counting fingers vision, vascularised corneal surface (figure 1C). Subtle conjunctivalisation of the left eye was noted at the site of previous limbal biopsy (figure 1D).

After much deliberation the informed choice was made to go ahead with deep anterior lamellar keratoplasty with amniotic coherence tomography, which was done in May 2010. The postoperative course was complicated by descemet's membrane detachment diagnosed on clinical examination and anterior segment optical coherence tomography

(OCT; figure 2A). The patient underwent descemetopexy with perfluoropropane (C_3F_8) gas and peripheral iridotomy 2 days later. This was partially successful as seen on the OCT with superior DM attached although the inferior remained detached (figure 2B). The patient was then treated conservatively and further review a month later revealed visual acuity of 20/30, clear graft with an attached DM (figures 1E and 2C).

TREATMENT

Following her keratoplasty she was treated with topical prednisolone drop 1% six times a day, ofloxacin four times a day and cyclopentolate three times a day. The antibiotic and cycloplegic drops were discontinued after 4 weeks and the steroid drops were tapered to once a day over 9 months.

OUTCOME AND FOLLOW-UP

She was subsequently reviewed on a 3–6 monthly basis and on her last review in March 2012 she had a best corrected visual acuity of 20/80, clear central graft and posterior subcapsular cataract. One particular point of interest in the final slit lamp photographs is that there appears to be a clear central optical zone surrounded by arrested peripheral conjunctivalisation (Figure 1F). There was no refractive change in the patient following limbal stem cell treatment with the left eye plano and the right eye refraction $-0.25/+0.75 \times 120$. The refraction did not change significantly during the time of her treatment at LVPEI. No studies have looked at the effect of stem cell treatment on the refraction of patients so far.

DISCUSSION

Ever since the publication of case reports of patients with clinically diagnosed limbal stem cell deficiency maintaining a central clear corneal epithelial island, the previously understood dogma of corneal epithelial homeostasis has been challenged.⁵ This is supported by mammalian studies that support the theory that the corneal limbus may not be the only location for the corneal stem cells and they are distributed throughout the surface.⁶ This

Figure 2 (A) This anterior segment OCT image demonstrates subtotal descemet's membrane detachment. (B) This is the appearance following descemetopexy on day 1 with an air bubble abutting the descmet's membrane superiorly and ongoing inferior detachment. (C) This OCT image demonstrates the spontaneous resolution of the descmet's membrane detachment and its complete reattachment.

model is again challenged by this case report as although we performed two surgeries to repopulate the corneal stem cells, clinical LSCD was still present. This however still led to a successful lamellar transplantation. Of note peripheral arrested conjunctivalisation was present with a clear central island, similar to the cases previously described.⁵

As the results of corneal transplantation (both lamellar and penetrating) are poor without initial surgery to repopulate the corneal stem cells in limbal stem cell deficiency, it is clear that a healthy population of stem cells must be present for the corneal graft to survive in this situation.^{4 7-9} We postulate that each successive CLET transferred some stem cells, although these were not able to repopulate entire surface in that particular microenvironment. Each CLET is also only a monolayer of cells, thought to be a proportion of limbal stem cells and transient amplifying cells (although the ratio is unknown). These may have improved the grade of limbal stem cell deficiency, although this was subclinical, better diagnostic tools may help to confirm this. Following this, the transplantation of corneal tissue included a multilayered epithelium and may even include some donor stem cells. This may have improved the ocular microenvironment enough to allow the stem cells to regenerate and repopulate the corneal surface. The removal of the corneal pannus (which appears like an opaque cornea with

stromal scarring, although reveals a clear stoma following removal) and replacement of epithelial stem cells gives rise to a remarkably clear cornea in successful cases. This may offer a new treatment strategy to those patients who were previously thought of as failed CLET and for whom precious donor cornea material would result in a poor outcome. Although we were hopeful of a positive outcome, we note in this case that the first and subsequent limbal stem cell treatments were clinically unsuccessful. She had recurrence of her corneal erosions with pain and her cornea was covered with conjunctival overgrowth. We know that younger patients tend to have worse outcomes with CLET even though the anatomy of the limbal niche is better preserved (confirmed with subgroup analysis of unpublished data LVPEI). This can also be seen in the publication of the case series undertaken by LVPEI.⁵ The reasons for this are not clear and it may be that the severity of the injury is greater in children with delayed presentation, or maybe due to the nature of the host immune response. The optimal timing between a CLET and corneal transplantation is not clear, although LVPEI usually wait at least 6 months before undertaking such treatment (although supportive surgery is usually performed acutely).

This case highlights the need for more diagnostic tools to be available and developed to help diagnose and grade the severity of limbal stem cell failure. It also highlights

that the mechanism by which CLET is able to result in repopulation of the patient's corneal stem cells is not fully understood and requires further research. With regard to the fate of the corneal stem cells some groups are starting to visualise the Pallasades of Vogt and limbal structures with OCT and confocal and also characterise affects of age on the structures of the niche.¹⁰ If these accessible imaging technologies could be combined with the successful labelling of transplanted cells as other specialties have done, we would be able to undertake in vivo tracking of transplanted cells to determine their fate and how best to optimise outcome.¹¹

Learning points

- ▶ The mechanism by which cultivated limbal stem cell transplantation works is poorly understood, although studies show that the majority of patients (>66%) achieve clinical success. Repeated transplantation leads to increased success rates (>75%).
- ▶ Subclinical improvement may take place on those patients previously labelled as treatment failures.
- ▶ Tracking corneal stem cells after they have been transplanted would help us to better understand the fate of these cells, and may help direct research in other specialties.

Competing interests None.

Patient consent Obtained.

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