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Rainer Sundmacher
Thomas Reinhard

Central corneolimbal transplantation under systemic ciclosporin A cover for severe limbal stem cell insufficiency

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R. Sundmacher · T. Reinhard (✉)
Department of Ophthalmology,
Heinrich Heine University,
Moorenstrasse 5, D-40225 Düsseldorf,
Germany
Tel. +49-211-311 7320;
Fax +49-211-311 6241

Abstract ● Background: Severe stem cell deficiencies uniformly lead to superficial conjunctivalization of corneal grafts with subsequent functional failure. We sought better long-term results by transplanting central corneolimbal grafts and simultaneously protecting the graft and its stem cells from immunological destruction by means of systemic administration of ciclosporin A. ● Patients and methods: In an ongoing pilot study, up to April 1995 20 eyes with stem cell dysfunctions of various etiology (e.g. chemical burn, ocular pseudopemphigoid, congenital aniridia) received eccentrically trephined fresh corneal grafts of 7.7–10.0 mm diameter. About one third of the circumference of the grafts contained limbal area. The mean age of the patients was 46.2 years (range 9–84 years). All patients received systemic ciclosporin A for at least 12 months. At present, the mean follow-up period is 9.6 months (mean 1–20.6 months). ● Results: Fourteen of 20 grafts (70%) have remained clear so far. Reasons for six graft failures were surface disorders in four eyes, immune reactions in one eye and surface disorders in combination with immune reactions in another eye. Ten of 20 grafts (50%) experienced severe surface disorders. In six eyes surface disorders were coincident with endothelial immune reactions, in four eyes they were not. In four of 20 grafts (20%) conjunctivalization was observed in front of the transplanted limbal area; in seven of 20 grafts (35%) conjunctivalization occurred only distant from the transplanted limbal stem cells. ● Conclusions: Our method of central corneolimbal transplantation with simultaneous protection of the transplanted stem cell population from immunological destruction by means of systemic ciclosporin A has been successful for 14 eyes with severe stem cell deficiencies up to 20.6 months postoperatively. This new treatment principle promises – for the first time – long-term rehabilitation for a majority of eyes with severe limbal stem cell deficiencies.

Introduction

In the past, immune reactions were the most important complication after penetrating keratoplasty [12]. With effective short-term topical and systemic steroid cover, their importance has gradually decreased in normal-risk penetrating keratoplasty [13]. In high-risk penetrating keratoplasty, their incidence has been reduced by systemic ciclosporin A prophylaxis [14]. In a further step, an even greater reduction in the frequency of immune reactions after penetrating high-risk keratoplasty will probably be brought about by a combination of systemic ciclosporin A administration and effective molecular genetic HLA typing and matching in the future [8].

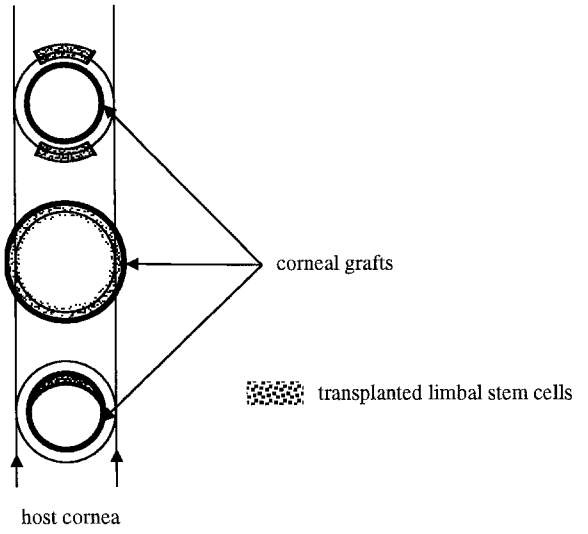

Fig. 1 *Top* Limbal transplantation, *middle* oversize graft with limbal stem cells, *bottom* central corneolimbal transplantation

With the more efficient suppression of immune reactions, surface disorders have become the leading complication after penetrating keratoplasty in general [10], and especially after high-risk penetrating keratoplasty under ciclosporin A cover [14]. It is important to differentiate between surface disorders without and those with severe limbal stem cell insufficiency. There have been several studies outlining the importance of limbal stem cells for the regeneration of the corneal epithelial surface [1, 5, 17]. *Without* severe limbal stem cell insufficiency, fresh grafts with excellent viable epithelium may provide high rates of graft survival [11]. Since the epithelium of fresh grafts will not survive longer than one year postoperatively, conjunctivalization of the graft will inevitably recur in cases *with* severe limbal stem cell insufficiency.

In the past, when the source of corneal epithelial regeneration was not known, autologous conjunctival transplantation was attempted [16], but this approach was abandoned when the corneal limbus was identified as the site of stem cells. Two possibilities for limbal stem cell transplantation are already known: separate auto- or homologous limbal stem cell transplantation with or without simultaneous penetrating keratoplasty [3, 4, 6, 15] (Fig. 1, top), and penetrating keratoplasty with homologous oversize grafts [2, 7] (Fig. 1, middle).

We have developed a third method for homologous limbal stem cell transplantation in a one-step procedure with simultaneous penetrating keratoplasty: central transplantation of an eccentrically trephined corneolimbal transplant.

Patients and methods

Since February 1993 we have performed central corneolimbal transplantations (Fig. 1, bottom) in 20 eyes of 13 male and six female patients with severe limbal stem cell deficiency or insufficiency. The diagnosis of limbal stem cell deficiency or insufficiency was made clinically by circular conjunctivalization of the cornea. Indications for surgery were: severe burns (16 eyes), pseudopemphigoid (two eyes), pannus in congenital aniridia (one eye) and limbal stem cell insufficiency of unknown origin (one eye). The mean age of the patients was 46.2 years (range 9–84 years), and the mean follow-up period was 9.6 months (range 1–20.6 months).

We used graft diameters between 7.7 and 10.0 mm. Fresh grafts with excellent epithelium excised 6–24 h after the donor's death and cultured for a maximum of 24 h in a short-term storage medium (Likorol) were used in all cases. The mean donor's age was 53.2 years (range 31–69 years). About one third of the grafts' circumference contained limbal area. Whenever possible the grafts were sutured with a double running cross-stitch nylon 10.0 suture. Only if the host's Bowman's layer had been severely destroyed were single 10.0 nylon sutures performed. If necessary, conventional cataract surgery, anterior and posterior synechiolysis, intraocular lens (IOL) explantation, anterior vitrectomy and transscleral fixation of posterior chamber IOLs were performed at the same time. Postoperatively, 60–100 mg of systemic fluocortolone was tapered over 3–4 weeks. Topical steroids (prednisolone-21-acetate 1% eye drops) were administered at doses of five to seven drops daily. Two drops daily were administered in the long term.

Systemic ciclosporin A administration was scheduled in all cases for at least 12 months. The drug was administered twice daily, aiming at trough levels of 100–150 ng/ml (monoclonal RIA/TDx, EDTA blood), determined 12 h after the last dose. The daily dosage of ciclosporin A varied between 150 and 500 mg. During the whole administration period intense nephrological monitoring was performed. If a graft failed irreversibly, ciclosporin A administration was stopped immediately.

If acute endothelial immune reactions occurred, we treated them systemically with 100 mg of fluocortolone and topically with up to ten drops of prednisolone-21-acetate 1% eye drops daily. Chronic endothelial and chronic stromal immune reactions were treated with five drops of prednisolone-21-acetate 1% daily.

Results

Fourteen (70%) of 20 grafts have remained clear so far. Causes for six graft failures were surface disorders (four eyes), immune reactions (one eye) and surface disorders combined with immune reactions (one eye).

After 10 (50%) of 20 corneolimbal transplantations, nine acute and two chronic endothelial immune reactions were observed. Ciclosporin A administration was stopped seven times: in the six patients with graft failure, and in a seventh patient who died three months postoperatively because of a cardiac infarct. All patients with clear grafts still receive systemic ciclosporin A.

Severe surface disorders (transplant erosion or ulcer) recurred after ten (50%) of 20 grafts (Fig. 2). In six eyes surface disorders coincided with endothelial immune reactions, in four eyes they did not. In four (20%) of 20 grafts conjunctivalization of the transplanted limbal area occurred; in seven (35%) of 20 grafts conjunctivalization

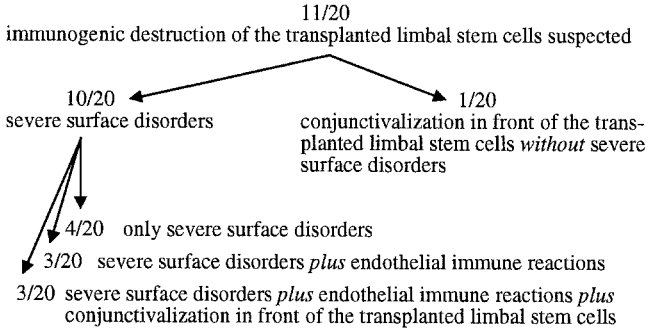

Fig. 2 Suspected immunogenic destruction of the transplanted limbal stem cells after 20 central corneolimbal transplantations

was observed only distant from the transplanted limbal stem cells (Figs. 3–6).

Discussion

With the present study we introduce a new method for rehabilitation of eyes with severe limbal stem cell deficiency or insufficiency. In these eyes, conventional corneal transplantation of normal-size central grafts is doomed to fail because of inevitable long-term conjunctivalization [11, 14]. Therefore, in this pilot study, 20 eyes were operated on by central corneolimbal transplantation.

Two threats for the graft were expected: (1) endothelial immune reactions and (2) surface disorders after immunological destruction of the transplanted limbal stem cells. Therefore, effective immunosuppression with systemic ciclosporin A for at least 1 year was judged to be absolutely necessary.

After ten central corneolimbal transplantations (50%) endothelial immune reactions occurred. This is a rather high rate compared with the 26.7% observed after conventional corneal transplantations in high-risk cases under systemic ciclosporin A [11]. A possible explanation for the latter high rate may be the transplantation of many more Langerhans cells and HLA-DR antigens [9], which result in a higher immunogenic stimulus to the host. Further, ciclosporin A blood levels between 100 and 150 ng/ml (EDTA blood, monoclonal RIA/TDx) were presumably not sufficient to block the enlarged immunogenic stimulus in these cases, and therefore higher levels may be necessary in the future to protect these central corneolimbal transplants.

An unsolved problem is the clinical diagnosis of immune reactions against limbal stem cells, which remains impossible. One may only indirectly suspect their occurrence from the acute or chronic development of severe surface disorders (Fig. 2). These were observed after ten (50%) of 20 central corneolimbal transplantations, which may indicate that in about half of the transplants we did not succeed in ultimately protecting the stem cells from immune destruction. Another diagnostic hint is surface disorders arising in combination with acute endothelial immune reactions. Such a combination occurred in six (30%) of 20 eyes. The diagnostic proof, of course, is definite conjunctivalization in front of the transplanted limbus. This was observed in four (20%) of 20 grafts, with severe surface disorders in three of them.

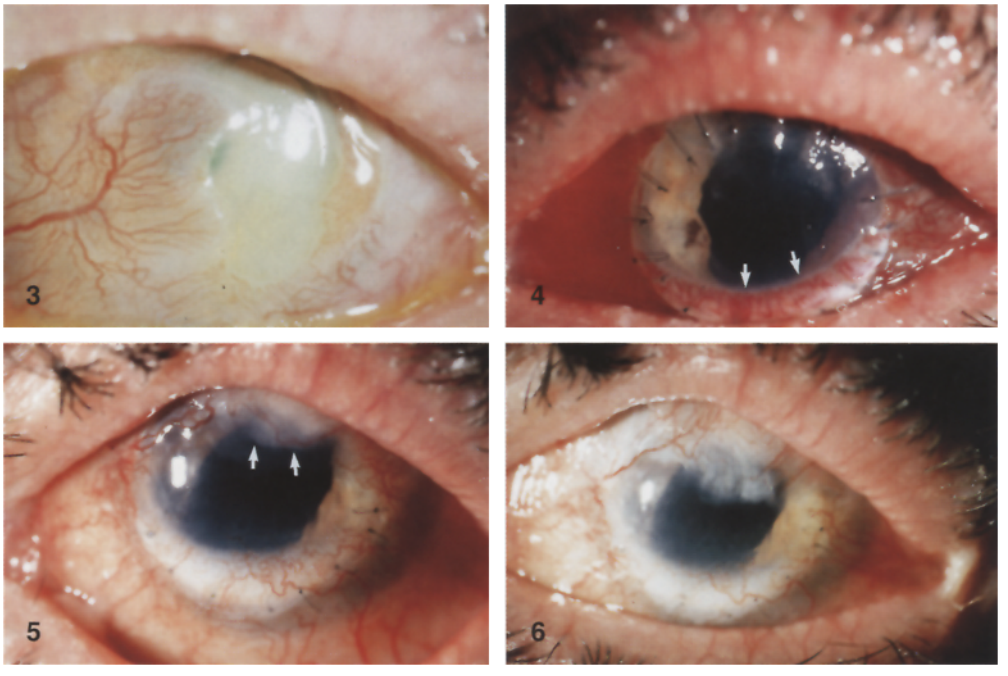

Fig. 3 Left eye of a 57-year-old female patient with pseudopemphigoid. Conventional surgery would have had no chance at all and would not have been attempted

Fig. 4 Same eye as in Fig. 3, shortly after central corneolimbal transplantation (limbus with *arrows*)

Fig. 5 Same eye as in Figs. 3 and 4, 8 months postoperatively. Conjunctivalization in progress opposite to the transplanted limbal stem cells (pannus with *arrows*)

Fig. 6 Same eye as in Figs. 3–5, 15 months postoperatively. Further progressive conjunctivalization opposite to the transplanted limbal stem cells with clear cornea in front of the limbal area and a vision of 0.3 cc in this only eye; a spectacular result for such an eye

Hence, in 11 eyes immunological destruction of the transplanted limbal stem cells had to be suspected, but only after four central corneolimbal transplantations could it be proven.

In summary, central corneolimbal transplantations under systemic ciclosporin A cover are a new and technically simple means to rehabilitate a significant proportion of eyes with severe limbal stem cell insufficiency, in this case for a mean period of 9.6 months so far. The best evidence for survival of stem cells is conjunctivalization distant from the transplanted limbus with, at the same time, absence of conjunctivalization in front of the limbal stem cells (Figs. 3–6). Immune reactions against endothelial cells and limbal stem cells are the main problem postoperatively and are presumably only partially inhibited by our current ciclosporin A regime. A longer period of systemic ciclosporin A prophylaxis with higher blood levels therefore seems to be necessary. Further progress may be expected from the combination of more effective HLA matching with systemic ciclosporin A prophylaxis.

References

1. Chen JY, Tseng SCG (1990) Corneal epithelial wound healing in partial limbal deficiency. Invest Ophthalmol Vis Sci 31: 1301–1314
2. Duke-Elder S, Leigh A (1965) Diseases of the outer eye. Corneal grafts. In: Duke-Elder S (ed) System of ophthalmology, vol 8. Kimpton, London, pp 648–66
3. Herman WK, Doughman, DJ, Lindstrom, RL (1983) Conjunctival autograft transplantation for unilateral ocular surface diseases. Ophthalmology 90: 1121–1126
4. Hoppeler T, Balogh T, Kalman A, Gloor B (1993) Autologe Limbustransplantation nach Verätzung. In: Gloor B, Hartmann C, Rochels R (eds) Kongreßband DGII. Springer, Berlin Heidelberg New York, pp 429–433
5. Huang AJW, Tseng SCG (1991) Corneal epithelial wound healing in the absence of limbal epithelium. Invest Ophthalmol Vis Sci 32: 96–105
6. Kenyon KR, Tseng SCG (1989) Limbal autograft transplantation for ocular surface disorders. Ophthalmology 96: 709–723
7. Kuckelkorn R, Redbrake C, Schrage NF, Reim M (1993) Keratoplastik mit 11–12 mm Durchmesser zur Versorgung von schwerstverätzten Augen. Ophthalmologe 90: 683–687
8. Mytilineos J, Scherer S, Opeltz G (1990) Comparison of RFLP DR beta and serological HLA-DR typing in 1500 individuals. Transplantation 50: 870–873
9. Pels E, Gaag R van der (1984/1985) HLA-A, B, C, and HLA-DR antigens and dendritic cells in fresh and organ culture preserved corneas. Cornea 3: 231–239
10. Redbrake C, Tympnert J, Reim M (1993) Komplikationen nach Keratoplastik. Ophthalmologe 90 [Suppl 1]: 147
11. Reinhard T, Sundmacher R, Heering P (1995) Systemic ciclosporin A in high-risk keratoplasty. Graefe's Arch Clin Exp Ophthalmol
12. Sundmacher R (1977) Immunreaktionen nach Keratoplastik. Klin Monatsbl Augenheilkd 171: 705–722
13. Sundmacher R, Stefansson A, Mackensen G (1983) Verlaufsbeobachtungen nach Keratoplastik. Fortschr Ophthalmol 80: 224–227
14. Sundmacher R, Reinhard T, Heering P (1992) Six years' experience with systemic cyclosporin A prophylaxis in high-risk perforating keratoplasty patients. Ger J Ophthalmol 1: 432–436
15. Tan DTH, Ficker LA, Buckley RJ (1994) Limbal transplantation. Invest Ophthalmol Vis Sci 35: 1913
16. Thoft RA (1977) Conjunctival transplantation. Arch Ophthalmol 95: 1425–1427
17. Tsai RJ, Sun T, Tseng SCG (1990) Comparison of limbal and conjunctival autograft transplantation in corneal surface reconstruction in rabbits. Ophthalmology 97: 446–455