Chapter 3

Validation of storage methods

Chapter 3.1

Clinical comparison of grafts stored in McCarey-Kaufman medium at 4° and in corneal organ culture at 31° C

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Abstract

Twenty-eight paired human corneas were preserved in minimal essential medium at 31°C and in McCarey-Kaufman medium at 4°C. These grafts were then transplanted in pairs of patients with keratoconus who were age matched as closely as possible. These pairs received donor corneas from the same donor, so for each pair the donor age and time from death to preservation were the same.

Visual acuity, central corneal thickness, and endothelial cell counts were compared.

During the 1- to 2-year study period, no statistically significant difference in visual acuity, corneal thickness, or endothelial cell density was found between grafts stored in minimal essential medium and those stored in McCarey-Kaufman medium.

Introduction

Preservation of human donor corneas is aimed at providing viable tissue for corneal transplantation. Advantages of preserving corneal tissue in a storage medium are an increased time from donor death to surgery, allowing convenient planning of the operation; quality control of the donor material; and conditioning of the tissue with respect to stromal swelling and prevention of microbial contamination.Preservation in McCarey-Kaufman (M-K) medium is widely used and allows storage up to 72 hours at 4°C.[1] Minimal essential medium (MEM) at 31°C allows tissue preservation up to 4 weeks.[2-5] This creates time for HLA matching and transportation over long distances to prospective donors selected through tissue typing. Others have also shown excellent preservation of endothelial viability in organ-cultured corneal tissue.[6,7] Medium-time corneal preservation by organ culture at 31°C has been the method of choice for storage of donor corneas at the Amsterdam Cornea Bank, the major distributor of HLA-matched and random tissue for grafting in the Netherlands.[8]

In this study, we report the results of a 2-year follow-up of a prospective comparison of corneal transplantation for keratoconus. The study used pairs of donor eyes from the same donor, of which one cornea was preserved in M-K medium at 4°C and the fellow cornea was preserved in tissue culture medium at 31°C.

Subjects and methods

Pairs of donor eyes, enucleated between 4 and 23 hours post mortem (mean time, 11.9 hours), were examined with a slit lamp for corneal scarring, endothelial changes, and any sign of previous ocular disease or surgery. Donor age was not a criterion to exclude tissue from use for transplantation.

All donors aged 6 years and older were acceptable. Donor eyes were rinsed with tap water and disinfected with 0.5% povidone-iodine solution. After neutralization of iodine and rinsing, the corneoscleral buttons were removed. One cornea was placed in M-K medium (tissue culture medium 199 with 5% dextran 40 [molecular weight, 40 000 d] and 0.1 mg/ml gentamicin) and kept at 4°C. The fellow eye was placed in modified MEM and stored at 31°C. This culture medium contained MEM with Earle's salts, L-glutamine and 20-mmol/l HEPES buffer (powder, Flow Laboratories, Irvine, Scotland); fetal calf serum (Flow Laboratories); penicillin, 100 U/ml (Gist-Brocades, Delft, the Netherlands); and streptomycin, 0.05 mg/ml (Gist-Brocades). The pH of the medium was 7.3.

A few days before transplantation, the cornea was transferred from this MEM medium to a similar solution, MEM transport, which is MEM supplemented with 5% dextran 500 (molecular weight, 500.000) (Pharmacia Laboratories, Uppsala, Sweden). Nystatin, 50 U/ml (Labaz, Maassluis, the Netherlands) was added. The media were aseptically prepared by membrane filtration (pore size, 0.22 µ) and stored at -20°C until use. The endothelium of the eyes, kept in the M-K medium, could not be evaluated before the corneas were

transplanted because facilities were lacking. Because both corneas were put in the media at the same time, the experiment did not allow evaluation of the endothelium of the MEM corneas either. Normally the endothelium is examined before preservation after staining with 0.3% trypan blue in 0.9% sodium chloride for 1 minute, rinsing in phosphate buffered saline, and subsequently immersing in 1.8% sucrose solution.

Five micrographs of the central cornea were obtained, and the number of cells was counted from the photographs. Endothelial cells were photographed by means of a noncontact specular microscope (Zeiss, Weesp, the Netherlands). The image was projected with the use of a standardized magnification, and cells were counted by hand with a grid corresponding to 0.05 mm^2 of surface. Cells straddling the grid frame were included along two sides and omitted on two other sides of the grid. At the end of the preservation period, when the cornea was transferred into the dextran-supplemented medium, the endothelium was evaluated.

The following criteria were adopted as indicators of the structural and functional integrity of the human corneal endothelium: (1) Descemet's folds covered with endothelium; (2) at least 2000 endothelial cells per square millimeter; (3) a regular cell mosaic; (4) swelling of the intercellular borders after exposure to a hypotonic 1.8% sucrose solution; (5) cell loss not exceeding 20% during a 30-day preservation period; and (6) clear-cut cell borders and a smooth appearance like the cells of fresh corneas.[5-8]

Donor age varied between 63 and 81 years (mean, 71.2 years). The time from death to preservation varied between 4 and 23 hours (mean, 11.9 hours). The time from enucleation to preservation was always 1 hour or less. Storage time in M-K medium varied between 2 and 48 hours (mean, 21.2 hours). Storage time in MEM varied between 24 and 120 hours (mean, 60 hours) and that in MEM transport between 96 and 216 hours (mean, 128.6 hours) (Table1).

Table 1 - Data on recipients and corneal grafts in pairs of patients with keratoconus

pair no	storage medium	age, years		death to preservation hours	M-K time hours	MEM time h		
		recipient	donor			total	storage time*)	transport time**)
1	M-K	40	76	12	24			
	MEM	44	76	12		192	72	120
2	M-K	28	70	11	19			
	MEM	48	70	11		168	48	120
3	M-K	44	65	17.5	44			
	MEM	26	65	17.5		192	72	120
4	M-K	17	64	22.5	2			
	MEM	48	64	22.5		144	48	96
5	M-K	25	76	5	22			
	MEM	27	76	5		216	96	120
6	M-K	30	78	23	19			
	MEM	20	78	23		192	24	168
7	M-K	42	63	16	24			
	MEM	27	63	16		240	24	216
8	M-K	38	80	4.5	23			
	MEM	39	80	4.5		192	24	168
9	M-K	34	81	4	20			
	MEM	23	81	4		216	72	144
10	M-K	49	70	9.5	18			
	MEM	45	70	9.5		144	48	96
11	M-K	25	72	12	12			
	MEM	26	72	12		168	48	120
12	M-K	53	67	7	20			
	MEM	22	67	7		168	72	96
13	M-K	19	68	12	48			
	MEM	39	68	12		240	120	120
14	M-K	29	67	10.5	2			
	MEM	49	67	10.5		168	72	96

M-K indicates McCarey-Kaufman medium; MEM, minimal essential medium

*) storage time in mimimal essential medium,

**) storage time in mimimal essential transport medium

The patients selected for the study were all consecutive individuals on our waiting list for penetrating keratoplasty for keratoconus, without further ocular disease or a history of eye abnormality. The pairs were selected as much as possible for age. There were a total of 28 patients in the study. The recipient age ranged from 17 to 53 years (average, 33.8 years) in the M-K group and 20 to 48 years (average, 34 years) in the MEM group (Table 1).

After having been preserved by a different method of corneal storage, the two corneas of each pair were transplanted into the two recipients with equal diameters of corneal grafts used for each pair. All transplantations were performed by the same surgeon using the same operative technique.

The donor cornea was punched from the endothelial side by means of a disposable guillotine-guided razor-blade trephine.[9] The recipient was trephined by a free-hand technique with a Grieshaber trephine.[10]
Grafts had a 0.25-mm larger diameter than the recipient trephine. The donor button was sutured with a combined interrupted (10-0 nylon) and running (11-0 nylon) method. Patients were examined at regular 6-week to 3-month intervals with the exception of those who were checked by their own ophthalmologists and were available only by calling them in on a special request.
Ultrasonic pachymetry was performed 6 months to 2 years after surgery. Endothelial specular microscopy was performed after 6 months to 2 years. Visual acuity was measured with the use of a Snellen chart with spectacle correction or contact lens at 6 months and 1 to 2 years after surgery.
For good comparison, visual acuities of the paired patients were measured either with contact lenses when both patients were fitted with lenses or, when only one or neither wore contact lenses, by spectacle refraction.

Results

At the conclusion of follow-up (range, 1 to 2.5 years), 27 of the 28 grafts were clear. One patient, who received a M-K preserved cornea, had herpetic keratitis (without having had a herpes simplex virus episode before) and rejection of the graft 6 months after transplantation; the graft subsequently failed, and this patient and his matched subject (pair 12) were excluded from further study.
On the first postoperative day, maximal corneal thickness was measured. In the M-K group, the corneal thickness varied between 0.560 and 0.700 mm (mean, 0.630 mm), and in the MEM group, the corneal thickness varied between 0.647 and 0.873 mm (mean, 0.748 mm).
After 12 to 24 months, the mean corneal thickness of the M-K-preserved corneas was 0.548 ± 0.053 mm and that of the MEM-preserved corneas, 0.565 ± 0.051 mm (Table 2). The difference was not statistically significant (Wilcoxon matched-pairs signed-rank test). Three pairs were excluded. In pair 2, both measurements were lacking, in pair 6 the M-K measurement was not done, and in pair 10 the MEM measurement was not done.

The endothelial cell count was measured after 6 to 24 months (Table 2). The mean endothelial cell count for the M-K group was 1966 ± 214/mm^2 and that for the MEM group, 1816 ± 288/mm^2. This difference was not statistically significant (Wilcoxon matched-pairs signed-rank test).

The mean visual acuity was measured 6 months and 12 to 24 months after the operation (Table 2). Three pairs were excluded because of amblyopia. Six months after the operation, the mean visual acuity in the M-K group was 0.77 ± 0.2 and that in the MEM group, 0.74 ± 0.2. This difference was not statistically significant (Wilcoxon matched-pairs signed-rank test). The visual acuity after 12 to 24 months was again not statistically different (0.98 ± 0.1 in the M-K group and 1.0 ± 0.2 in the MEM group).

Table 2 – Characteristics of pairs of patients with keratoconus after perforating keratoplasty

pair no	*storage medium*	*months post operative*	*cell density cells / mm^2*		*corneal thickness mm*	*postoperative visual acuity*	
			preoperative	*postoperative*		*6 months*	*1-2 years*
1	M-K	23		1780	537	1.0	1.0
	MEM	23	2600	1900	533	0.5(A)	0.5(A)
2	M-K	22		2053	586	0.8	0.8
	MEM	22	3000	1660	550	0.8	1.2
3	M-K	23		2050		0.9	1.0
	MEM	23	2600	1950		0.9	1.0
4	M-K	24		1800	537	0.8	1.2
	MEM	24	2700	1380	525	0.3(A)	0.3(A)
5	M-K	17		2320	500	0.5	1.0
	MEM	17	2700	1980	615	0.8	1.2
6	M-K	23		2120		0.6	1.0
	MEM	23	2900	1650	635	0.5	1.0
7	M-K	15		1825	556	0.6	0.9
	MEM	15	2500	1440	592	0.4	1.0
8	M-K	14		2060	640	0.5	1.2
	MEM	14		1460	665	0.8	0.8
9	M-K	13		2200	546	0.8	0.9
	MEM	13	2700	2300	581	0.8	1.2
10	M-K	11		1660	516	0.5(A)	0.6(A)
	MEM	11		2040		0.4	1.0
11	M-K	7		1940	482	1.0	1.0
	MEM	7	2600	2050	520	0.8	1.0
13	M-K	8		2130	483	1.0	1.0
	MEM	8	2700	1700	497	0.7	0.7
14	M-K	11		1620	613	1.0	1.0
	MEM	11	2400	2100	580	0.9	0.9

M-K = McCarey-Kaufman medium, MEM = mimimal essential medium, A = amblyopia
Patient 12 had herpetic keratitis and was excluded from the study

Comment

The short-term preservation of donor corneas at 4°C in M-K medium is the most frequently used preservation. Long-term organ culture at 31° to 34°C is less frequently used but has many advantages, because there is additional time for convenient planning of the operation, quality control of the donor material, and conditioning of the tissue with respect to stromal swelling and prevention of microbial contamination.

The tissue culture medium used in the Netherlands does not contain dextran. Although a direct effect of dextran on endothelial cell loss and endothelial viability was not definitely demonstrated, dextran has an indirect effect on the quality of the corneas used for transplantation. Therefore, the use of dextran is avoided as much as possible in the Netherlands. Medium with 5% dextran is used only for deswelling of the corneas before transplantation. The storage time in this medium is limited to 7 days to prevent excessive uptake of dextran.[11,12]

During the period under study here (1 to 2 years), no statistically significant difference in visual acuity, corneal thickness, or endothelial cell density was found between M-K- and MEM stored corneal grafts.

In an earlier study in our hospital, a difference was found in visual acuity between the M-K group and the MEM group, but in that study the pairs had a variety of diagnoses and were not evaluated at exactly the same time as in this study.[13]

The maximal corneal thickness was found during the first postoperative days and was different between the two groups (M-K medium, 0.700 mm; MEM, 0.873 mm), as we could expect from earlier studies. However, we do not have values for all the pairs. We know from earlier studies that these values could not be applied as predictors of the final corneal thickness because the final thickness was reached only after 1 year.[3] The central corneal thickness, found after 1 year, was comparable with the findings of others;[4,14,15] no statistically significant difference was found between the pairs of the two groups.

Graft survival was 92.9% in the M-K group and 100% in the MEM group. In another study, a better graft survival with donor corneas preserved in MEM than in M-K was found.[16] However, these patients were not paired as in our study.

No statistically significant difference in mean endothelial cell count was found between the M-K and MEM groups. In the absence of the preoperative corneal endothelial cell counts, we could only compare absolute values of postoperative cell counts of the pairs of patients with keratoconus. Because of the transport of the MEM cornea, the real preoperative endothelial cell densities (Table 2) can never be higher for the MEM-preserved corneas than for the M-K-preserved corneas.

Studies in which MEM preservation is compared with other preservation methods are few. This study is the first report, to our knowledge, in which the diagnosis was the same for all the patients, and the pairs received corneal transplants from the same donor. All measurements were done for the pairs at the same postoperative time, giving an excellent opportunity to compare preservation methods clinically.

As we know from earlier studies, the mean cell density decreases for 3 years after transplantation.[17] We plan to repeat our measurements after that time because by that time a stable situation has been reached, especially for the endothelial cell count.

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