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Suspension made with amniotic membrane: Clinical trial

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PURPOSE. To investigate if a suspension made with amniotic membrane could have a beneficial effect on ocular surface diseases.

METHODS. In the Imola branch of the Eye Bank of Emilia Romagna, the authors prepared a suspension containing homogenized amniotic membrane previously conserved at –80 degrees Celsius. Subsequently, the authors gave this preparation to 21 patients: 8 had undergone lamellar keratoplasty, 4 had undergone penetrating keratoplasty, 2 had undergone photorefractive keratectomy with a delay of epithelialization, 3 had neurotrophic corneal ulcers, 2 had corneal burning, 1 had torpid corneal ulcer, and 1 had Sjögren syndrome. Each patient had been treated with conventional therapy for at least, 4 months without any clinical improvement. In this sample of eyes the authors evaluated the transparency and integrity of epithelium before and after the therapy by means of a fluorescein staining test, examining the area of epithelial defect as well as the phlogistic situation and the symptoms referred by patients. Nine eyes from this group of patients were studied by impression cytology before and after 3 months of use of suspension. The follow-up was 5 months of once-weekly visits.

RESULTS. In all patients, after 15 to 30 days the corneas became negative to fluorescein staining test and the epithelium seemed more complete and regular, there was an evident decrease of phlogistic situation in the conjunctiva, and an improvement of symptoms was referred by patients. The situation was stable during the whole follow-up. No side effects were noted. The impression cytology repeated 3 months after the treatment showed a significant corneal recovery of the cytologic situation with an important decrease of CK19+ cells on the corneal surface.

CONCLUSIONS. This new therapy, which is less traumatic than an implant of amniotic membrane, is safe, and can be repeated for a long period, could help patients with corneal superficial defects. (Eur J Ophthalmol 2005; 15: 441-5)

KEY WORDS. Amniotic membrane suspension, Corneal surface disease, Growth factors

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INTRODUCTION

Amniotic membrane obtained from cesarean deliveries is prepared and cryopreserved under sterile conditions and can be sutured onto the ocular surface. Amniotic membrane-covered surfaces have been shown to induce rapid re-epithelialization (in 2 to 4 weeks) to a smooth and wettable surface and reduce inflammation, vascularization, and scarring, thus allowing successful surface reconstruction. For partial limbal deficiency with superficial involvement, amniotic membrane transplantation alone has been shown to be sufficient and superior to autograft limbal transplantation because there is no need to administer cyclosporine. For total limbal deficiency,

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additional autograft limbal transplantation is needed, and amniotic membrane transplantation has been shown to enhance successful engraftment of autograft limbal transplantation by preparing the perilimbal stroma and reducing inflammation and vascularization. Nowadays we know that amniotic membrane transplantation is an excellent treatment for many ocular surface diseases. The exact mechanisms by which amniotic membrane delivers its beneficial effect on the ocular surface are still being investigated but human amniotic membrane has been shown to contain collagen type III and V; it also contains collagen types IV and VII, similar to corneal epithelial basement membrane, as well as fibronectin and laminin (1, 2). Additionally, it contains fibroblast and other growth factors (3, 4).

TABLE I - RATE OF HEALING IN THE GROUP OF 21 EXAMINED PATIENTS

Area of epithelial defect	Percentage decrease, median (range)	p value
1st month		
Day 7	59.0 (-47.0 to 87.7)	0.019
Day 14	78.8 (-29.1 to 100.0)	0.023
Day 21	79.4 (-65.6 to 89.9)	0.016
Day 28	100.0 (-98.7 to 100.0)	0.009
2nd month		
Day 7	100.0 (-98.7 to 100.0)	0.009
Day 14	100.0 (-98.7 to 100.0)	0.009
Day 21	100.0 (-98.7 to 100.0)	0.009
Day 28	100.0 (-98.7 to 100.0)	0.009
3rd month		
Day 7	100.0 (-98.7 to 100.0)	0.009
Day 14	100.0 (-98.7 to 100.0)	0.009
Day 21	100.0 (-98.7 to 100.0)	0.009
Day 28	100.0 (-98.7 to 100.0)	0.009
4th month		
Day 7	100.0 (-98.7 to 100.0)	0.009
Day 14	100.0 (-98.7 to 100.0)	0.009
Day 21	100.0 (-98.7 to 100.0)	0.009
Day 28	100.0 (-98.7 to 100.0)	0.009
5th month		
Day 7	100.0 (-98.7 to 100.0)	0.009
Day 14	100.0 (-98.7 to 100.0)	0.009
Day 21	100.0 (-98.7 to 100.0)	0.009
Day 28	100.0 (-98.7 to 100.0)	0.009

TABLE II - STUDY OF LIMBAL SITUATION BEFORE AND AFTER THE USE OF THE SUSPENSION WITH IMPRESSION CYTOLOGY

	Before the use of the suspension	3 months after the use of the suspension
1 Sjögren syndrome	No LSCD	No LSCD
1 Caustic burning	Moderate LSCD	Light LSCD
1 Caustic burning	Moderate LSCD	Light LSCD
1 Penetrating keratoplasty	No LSCD	No LSCD
1 Penetrating keratoplasty	Mild LSCD	Light LSCD
1 Penetrating keratoplasty	Mild LSCD	No LSCD
1 Neurotrophic keratopathy	Severe LSCD	Light LSCD
1 Neurotrophic keratopathy	Moderate LSCD	Light LSCD
1 Neurotrophic keratopathy	Severe LSCD	Moderate LSCD

LSCD = Limbal stem cell deficiency

We know that amniotic membrane modulates levels of cytokines and growth factors and has also been shown to have unique properties, including pain reducing, fibrosis suppressing, antibacterial, and wound protecting features (5-12). It is unclear whether amniotic membrane promotes limbal stem cells proliferation (13, 14). Many studies have shown that amniotic membrane promotes epithelialization of the corneal surface and this helps to restore the ocular surface (3). A recent study has discovered that preserved amniotic membrane at –80 degrees C expresses mRNAs for a number of growth factors and contains several growth factor proteins that might benefit the epithelialization: high levels of EGF, TGF-alfa, KGF, HGF, bFGF, TGF-beta1, -beta2, -beta3 mRNA, and proteins have been found in amniotic membrane, therefore the research suggests an epithelial origin for these growth factors (3). Additionally there are several studies that demonstrate the important effect of the same growth factors in stimulating and modulating epithelialization after corneal surface damage (15-18).

We hypothesized that a suspension made with amniotic membrane could cause a similar effect on corneal disease, giving a prominent role to growth factors in the beneficial effect of amniotic membrane transplantation.

METHODS

In the Imola branch of the Eye Bank of Emilia Romagna, we prepared a suspension using 5 cc of BSS, 1 cc of antibiotic, and a homogenized patch (with the dimension of 2 cmm^2) of amniotic membrane preserved at –80 degrees Celsius. We tried to use different quantities of amniotic membrane and we discovered that using a patch of 2 cmm^2 we could obtain a fluid, transparent, and stable suspension without deposits. We used the Heidolph homogenizer (DIAX 100). Then we preserved this preparation at a temperature of –20 degrees Celsius.

Subsequently, we gave this suspension to 21 patients: 8 had undergone lamellar keratoplasty and showed signs of initial melting and deficit in the re-epithelialization, 4 had undergone penetrating keratoplasty and showed noninfected erosive chronic ulcers near limbus with important epithelial defects, 2 had undergone photorefractive keratectomy (PRK) with recurrent epithelial defects, 3 had neurotrophic corneal ulcers, 2 had corneal and caustic burning, 1 had torpid corneal ulcer, and 1 had Sjögren syndrome. Each cornea showed zones with positive fluorescein staining test. Epithelial defects measuring at least 2 mm in linear dimension were included. Serial measures of the size of the epithelial defects, namely, estimation of the area of an equivalent rectangle obtained using the largest linear dimension of the epithelial defect and its largest possible perpendicular within the confines of the defect, were done at start of therapy and during the follow-up.

Each of these patients had received the following treatments for at least 4 months without any clinical improvement:

- Topical drugs with vitamin A with and without blindfold
- Therapeutic contact lens
- Preparation made with autologous serum
- Artificial tears
- Specific vitamins.

In the patients with Sjögren syndrome and with corneal and caustic burning, we did punctual occlusion.

The average age of this group was 57±21 years, and there were 9 females and 10 males.

We gave the amniotic membrane suspension with the following posology: two drops six times a day. The suspension lasted 2 weeks; then it was changed with a new one.

Each patient was examined once a week for 5 months with a minimum follow-up of 3 and maximum of 6 months. In these patients we studied the zones positive to fluorescein staining test, the integrity and transparency of epithelium, the phlogistic situation of ocular surface, and the ocular discomfort referred by patients before and after treatment. Nine eyes from this group of patients were studied by impression cytology before and after 3 months of use of suspension (1 eye had Sjögren syndrome, 2 caustic burning, 3 had been operated on for penetrating keratoplasty, 3 had neurotrophic keratopathy). To classify the different levels of limbal stem cell deficiency (LSCD), we used the score proposed by Dionisi et al: they scored diffuse LSCD as mild (<25% of CK19-positive cells), moderate (25 to 50% of CK19-positive cells), and severe (>50% of CK19-positive cells) (19).

RESULTS

After 14 days, fluorescein staining test of cornea was negative in 12 patients (1 Sjögren syndrome, 2 penetrating keratoplasty, 3 lamellar keratoplasty, 2 neurotrophic ulcer, 2 corneal caustic burning, 2 PRK), and after 28 days

the other 9 patients showed the same situation. The healing rate is shown in Table I.

During the follow-up the situation improved because the eye showed evident signals of reduction of inflammation and an increase of epithelial integrity and transparency.

This result has been stable for the whole period of observation; we did not find side effects.

Moreover, of the nine patients studied with impression cytology, seven showed a mild or moderate diffuse LSCD before treatment; the examination repeated 3 months after the treatment with the suspension showed a better situation in all eyes, with a significant corneal recovery of the cytologic situation, and with an important decrease of CK19+ cells on the corneal surface (Tab. II).

Additionally, when interviewed, all the patients referred an evident relief since the first week of therapy.

We noted that upon stopping the suspension, the ulcers reappeared in two of the corneas with neurotrophic ulcers.

CONCLUSIONS

Our study indicates that this preparation is efficient in corneal restoration in different surface diseases.

We hypothesize that the high healing rate is based on the growth factors present in this suspension that are demonstrated to be essential in corneal wound healing and are well represented in amniotic membrane. A study is being carried out to demonstrate their presence in our suspension.

This new kind of therapy has several advantages:

- It is easy to prepare for every eye bank
- It is safe, with no side effects
- It is less traumatic for the eye than an implant of amniotic membrane
- It can be used for a long period of time (the amniotic membrane transplantation lasts a limited time). This therapy lacks the contact lens effect of amniotic membrane implantation. This simple preparation has the potential to aid ophthalmologists in curing a number of eye surface diseases.

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REFERENCES

1. Modesti A, Scarpa S, D'Orazi G. Localization of type IV and V collagens in the stroma of human amnion. Prog Clin Biol Res 1989; 296: 459-63.
2. Fukada K, Chikama T, Nakamura M, et al. Differential distribution of subchains of the basement membrane components type IV collagen and laminin among the amniotic membrane, cornea, and conjunctiva. Cornea 1999; 18: 73-9.
3. Koizumi NJ, Inatomi TJ, Sotozono CJ, Fullwood NJ, Quantock AJ, Kinoshita S. Growth factor mRNA and protein in preserved human amniotic membrane. Curr Eye Res 2000; 20: 173-7.
4. Sato H, Shimazaki J, Shinozaki N. Role of growth factors for ocular surface reconstruction after amniotic membrane transplantation. Invest Ophthalmol Vis Sci 1998; 39: S428.
4. Adinolfi M, Akle CA, McColl I. Expression of HLA antigens, beta2-microglobulin and enzymes by human amniotic cells. Nature 1982; 295: 325-7.
6. Tseng SCG, Esparta EM, Kawakita T, et al. How does amniotic membrane work? The Ocular Surface 2004; 2: 177-87.
7. Sippel KC, Ma J, Foster CS. Amniotic membrane surgery. Curr Opin Ophthalmol 2001; 12: 269-81.
8. Kruse Fe, Joussen AM, Rohrschneider K, et al. Cryopreserved human amniotic membrane for ocular surface reconstruction. Graefes Arch Clin Exp Ophthalmol 2000; 238: 68-75.
9. Trelford JD, Trelford-Sauder M. The amnion in surgery, past and present. Am J Obstet Gynecol 1979; 134: 833-45.
10. Colocho G, Graham WP, Greene AE, et al. Human amniotic membrane as a physiologic wound dressing. Arch Surg 1974; 109: 370-3.
11. Subrahmanyam M. Amniotic membrane as a cover for microskin graft. Br J Plast Surg 1995; 48: 477-8.
12. Shimazaki J, Yang HY, Tsubota K. Amniotic membrane transplantation for acute chemical and thermal burns. Ophthalmology 1997; 104: 2068-76.
13. Tseng SCG, Prabhasawat P, Barton K, et al. Amniotic membrane transplantation of autologous limbal epithelial cells. N Engl J Med 2000; 343: 86-93.

14. Wilson SE, Chen L, Mohan RR, Liang Q, Liu J. Expression of HGF, KGF, EGF and receptor messenger RNA following corneal epithelial wounding. Exp Eye Res 1999; 68: 377-97.
15. Woo H-M, Kim MS, Kweon O-K, Kim D-Y, Nam T-C, Kim JH. Effect of amniotic membrane on epithelial wound healing and stromal remodelling after excimer laser keratectomy in rabbit cornea. Br J Ophthalmol 2001; 85: 345-9.
16. Sotozono C, Kinoshita S, Kita M, et al. Paracrine role of keratinocyte growth factor in rabbit cornea epithelial cell growth. Exp Eye Res 1994; 59: 385-92.
17. Sotozono C, Inatomi T, Nakamura M, Kinoshita S. Keratinocyte growth factor accelerates corneal epithelial wound healing in vivo. Invest Ophtalmol Vis Sci 1995; 36: 1524-9.
18. Tseng SC, Li DQ, Ma X. Suppression of transforming growth factor-beta isoforms, TGF-beta receptors type II, and myofibroblast differentiation in cultured human corneal and limbal fibroblast by amniotic membrane matrix. J Cell Physiol 1999; 179: 325-35.
19. Dionisi PM, Rama P, Fasolo A, Ponzin D. Analysis of limbal stem cell deficiency by corneal impression cytology. Cornea 2003; 22: 1-6.