ISMB

TUMS

Effect of Amniotic Membrane Combined with Ciprofloxacin in Curing the Primary Stages of Pseudomonal Keratitis

Mohammad Mehdi Soltan Dallal [1,2], Farhad Nikkhahi [1], Saber Molaei [3], Kazem Hosseini [4], Zohreh Kalafi [1], Ahad khoshzaban [3], Abdolaziz Rastegar Lari [2], Ahmad Kheirkhah [3,4], Mohammad Kazem Sharifi Yazdi [5]*

[1] *Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran, IR Iran*
[2] *Antibiotic Resistant Research Center, Tehran University of Medical Sciences, Tehran, IR Iran*
[3] *Eye Research Center, Farabi Eye Hospital, Tehran, IR Iran*
[4] *Quality Control Manager of Iranian Tissue Bank Research & Preparation Center, Stem Cells Preparation Unit., Tehran, IR Iran*
[5] *Department of Medical Laboratory Sciences, School of Paramedicene, Tehran University of Medical Sciences, Tehran, IR Iran*

ARTICLE INFO

Article type:
Original Article

Article history:
Received: 21 Apr 2012
Revised: 29 May 2012
Accepted: 07 Aug 2012

Keywords:
Pseudomonas aeruginosa
Amnion
Ciprofloxacin
Keratitis
Rabbits

ABSTRACT

***Background*:** Keratitis caused by *Pseudomonas aeruginosa* is often resulted in severe corneal ulcers and perforation, which leads to losses of vision. Human amniotic membrane (HAM) forms the inner wall of the membranous sac which surrounds and protects the embryo during gestation. The purpose of this study was to evaluate the effectiveness of the amniotic membrane's healing in rabbits with pseudomonas keratitis.

***Methods*:** In total 14 rabbits divided in 2 groups of: 1 as Control and 2 as experimental amniotic membrane combined with ciprofloxacin. A 0.05 ml suspension of *Pseudomonas aeruginosa ATCC* 27853 was injected into rabbit's corneal stroma, with no interference in control group. In the second group, the amniotic membrane in pieces of 1.5 × 1.5 cm transplanted to the entire corneal surface by eight interrupted 10.0 nylon sutures. In the first day ciprofloxacin drop was injected to the second group every 30 minutes and through second to seventh days every 2 hours. The results of perforation in cornea and the amount of infiltration were registered.

***Results*:** The results showed that amniotic membrane transplantation (AMT) + ciprofloxacin group had 0% perforation and the control group 85.6%. Average infiltrations were 5 mm in AMT + ciprofloxacin groups and 23.75 mm in control.

***Conclusion*:** The use of amniotic membrane with ciprofloxacin was effective in prevention of cornea perforation and controlling the process of pseudomonal keratitis remission. The improvement of inflammation rapidly happened in ciprofloxacin + AMT group.

- ***Please cite this paper as:*** Soltan Dallal MM, Nikkhahi F, Molaei S, Hosseini K, Kalafi Z, Khoshzaban A, Rastegar Lari A, Kheirkhah A, Sharifi Yazdi MK, Effect of Amniotic Membrane Combined with Ciprofloxacin in Curing the Primary Stages of Pseudomonal Keratitis. *J Med Bacteriol.* 2012; **1** (1, 2): pp. 31-37.

* *Corresponding Author*: Mohammad Mehdi Soltan Dallal, PhD., Division of Microbiology, Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences (TUMS), Tehran, IR Iran, Antibiotic Resistant Research Center, Tehran University of Medical Sciences, Tehran, IR Iran. *Tel:* +98 21 88992971, *Fax:* +98 21 88954913, *E-mail*: soltinarad34@yahoo.com

Introduction

Human amniotic membrane (HAM) forms the inner wall of the membranous sac that surrounds and protects the embryo during gestation. HAM can reduce scarring and inflammation, enhance wound healing, and to serve as a scaffold for cell proliferation and differentiation due to its antimicrobial properties. It consists of a single layer of ectodermally derived columnar epithelial cells attached to a basement membrane with an underlying layer of mesenchyme (1). Amniotic membrane transplantation (AMT) is widely used in various ocular surface diseases such as neurotrophic keratitis and persistent epithelial defects (2, 3), band keratopathy (4), bullous keratopathy (5, 6), after excimer laser photorefractive keratectomy (7, 8), after the excision of a conjunctival mass (9, 10), pterygium (11, 12), ocular surface reconstruction in symblepharon (12, 14), acute chemical injury (15, 16) and chronic limbal deficiency (17, 18). When used as a graft in epithelial side up, AM is expected to become incorporated in the recipient tissue. When used as a patch (epithelial side down), it works as a biological bandage as a cover for a limited duration or a combination of these.

The use of AM has been also suggested in the treatment of infectious keratitis because of its intrinsic anti-infective properties probably mediated by its anti-inflammatory effects or AM may act as a long-term drug delivery system (19-21).

The purpose of this study was to evaluate effect of Amniotic membrane combined with ciprofloxacin in curing the primary stages of pseudomonal keratitis.

Methods

In total 14 rabbits with the average weight of 1.5-2 kg prepared from *Razi Vaccine and Serum Research Institute*. The amniotic membrane was prepared according to Dr. T Seng and Kim method (27). Briefly, human placenta was obtained Imam Khomeini Hospital after an elective caesarean section in a woman who was serologically negative for human immunodeficiency virus, hepatitis B, C and syphilis. Under a lamellar flow hood, the placenta was first washed free from blood clots with sterile saline. The inner amniotic membrane was separated from the rest of the chorion by blunt dissection and flattened on a nitrocellulose membrane. The membrane with the filter was washed three times with phosphate buffered saline (PBS) containing 50 µg/ml penicillin, 50 µg/ml streptomycin and 2.5 µg/ml amphotericin B and it was added to M199 medium containing streptomycin, cloxacillin, cetriaxone and amphotericin B for 24 hours and finally packed in pieces of 1.5 × 1.5 in three sterilized nylons and stored in -80ºC in freezer. Twenty eight rabbits were divided in 2 groups, one as control and second as amniotic membrane combined with ciprofloxacin. The rabbits were anesthetized with intramuscular injection of ketamine hydrochloride (30 mg/kg) and xylazine hydrochloride (5 mg/kg) and then a drop of tetracaine HCL 0.5% was applied to the right eye of rabbits. Fifty microliter suspension of *Pseudomonas aeruginosa* ATCC 27853 was

injected into corneal stroma with a sterile 30G needle connected to a micro syringe, using an operating microscope. The experimental keratitis was allowed to proceed untreated for 20 hours. There was no interference in control group. In two groups, the amniotic membrane in pieces of 1.5 × 1.5 cm transplanted to the entire corneal surface by eight interrupted 10.0 nylon sutures. In the first day, ciprofloxacin was dropped to the second group every 30 minutes and continued in the second to seventh day every 2 hours. The results were registered with aspect to perforation in cornea and the amount of size infiltration by the use of image J software.

Results

The results were recorded as clinical signs in first, third and seventh days. During the first 20 hours after injection of *Pseudomonas aeruginosa* a white opacity was appeared in all rabbits. Rabbits had corneal ulcers and in second day the conjunctiva was markedly hyperemic in four groups. At the end of the first week Hypopyon formation was observed in five cases in AMT group, and corneal perforation was occurred in four cases in the control group but no case was observed in amniotic membrane + ciprofloxacin group.

Control

First day

Average infiltration of 2.25 mm with corneal opacity and without epithelial defect was observed.

Third day

Average infiltration of 7.01 mm with corneal opacity, epithelial defect and descemetocele was observed.

Seventh day

Average infiltration of 23.75 mm with corneal opacity and epithelial defect was observed.

Ciprofloxacin + AMT

First day

Average infiltration was 2.6 mm without epithelial defect.

Third day

Average infiltration was 4 mm without epithelial defect.

Seventh day

Average infiltration was 5 mm without epithelial defect and scar.

Discussion

Few studies have investigated the effectiveness of AMT in the surgical treatment of severe infectious keratitis with corneal ulceration or perforation (22-25).

The main advantages of AMT in the treatment of bacterial keratitis are the epithelial bandage properties, which allow the early use of topical steroids, the anti-inflammatory and anti-scarring effects of the AM, the promotion of epithelialization and the possible benefits of a direct antimicrobial role of the AM (26).

AMT has been widely employed since Kim and Tseng first used preserved human AM as engraft in rabbit eyes in 1995 (27). Since then, modifications in the tissue processing techniques have evolved over the years.

Table 1. Results of 2 groups in examination *Pseudomonas* keratitis

Results	AMT + ciprofloxacin (mm)	Control (mm)
Perforation	(0%)0	(85.7%)6
Infiltration	5	23.75

Table 2. Comparison of control with AMT + ciprofloxacin

Group		*p*-value
AMT + ciprofloxacin	Control	$p < 0.05$

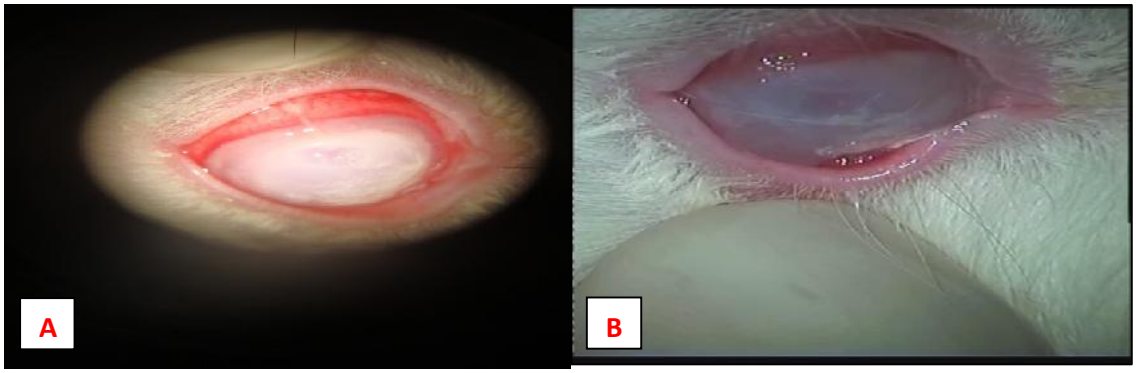

Figure 1. Generally the cornea has infiltration in central part; an area with the size of 6 mm had descemeto cell and progress toward causing perforation in cornea control group (picture A). In picture B which shows ciprofloxacin + AMT group conjunctiva inflammation is less than control group and in cornea examination the amount of infiltration is 5 mm and the decrease of opacity cornea is quite visible.

These studies found AMT to be effective in treating neurotrophic ulcer, inflammatory corneal ulcer, bulbous keratopathy, inflammatory or non-inflammatory sclera ulcer and as an adjuvant treatment of pterygium excision. The basement membrane of an AM promotes epithelial growth and differentiation, reinforces the adhesion of basal epithelial cells and prevents epithelial apoptosis.

The stroma matrix suppresses TGF-b signaling, proliferation and fibroblastic differentiation of normal human corneal and limbal fibroblasts, thereby inhibiting the unwanted production of extracellular matrix and scarring (6). These properties have made the AM as an ideal reconstructive substrate for repairing persistent epithelial defects, corneal ulcers (2), conjunctival defect (9, 10), chemical or thermal injury (15, 28) and limbal cell deficiency (16, 17). The application of AM in the treatment of corneal perforation and scleromalacia has been also reported (29, 30). The human amniotic membrane (AM) possesses anti-inflammatory, antifibrotic and antiangiogenic properties, and these attributes makes it ideal for ocular surface reconstruction procedures. (30, 31) In addition, the AM also has antimicrobial properties partly due to its anti-inflammatory effects and also due to secretion of Elafin and secretory leucocyte proteinase inhibitor, both of which have antimicrobial activity and act as components of the innate immune system (32, 33). It also contains Cystatin E, an analogue of cysteine proteinase inhibitor, which has complementary antiviral properties (34). In spite of this, AM transplantation (AMT) is reserved for cases of post infection ulcers after an appropriate period of anti-infective treatment when clinical signs are improving (36). This is because of anti-infective properties of AM which are non-

specific and not considered to be effective enough in acute infective keratitis. This is the reasoning behind the concept of fortifying AM with antimicrobial drugs to make it a therapeutic modality in the setting of active infections of the cornea. Antibiotic-impregnated medical devices such as catheters, bone and cardiac implants have been used for over a decade (35, 36). Various studies have shown the potential of such an approach in vascular surgery and arthroplasty, where they appear effective in reducing the risk of bloodstream infections or in limiting deep wound infections (37, 38).

Conclusion

Recovery process of inflammation in ciprofloxacin + AMT group occurred rapidly. The results of this study showed that use of Amniotic membrane with ciprofloxacin was effective in process of preventing cornea perforation and controlling remission of pseudomonal keratitis.

Acknowledgment

This work was supported by the Vice-Chancellor for Research grant (No. 10793) of Tehran University of Medical Sciences (Tehran, IR Iran).

References

1. Van Herendael BJ, Oberti C, Brosens I. Microanatomy of the human amniotic membranes. A light microscopic, transmission, and scanning electron microscopic study. *Am J Obstet Gynecol* 1978; **131** (8): 872-80.
2. Prabhasawat P, Tesavibul N, Komolsuradej W. Single and multilayer amniotic membrane transplantation for persistent corneal epithelial defect with and without stromal thinning and perforation. *Br J Ophthalmol* 2001; **85** (12): 1455-63.
3. Shin KS, Chung IY, Seo SW. The effect of amniotic membrane transplantation for corneal ulcer and ocular surface diseases. *J Korean Ophthalmol Soc* 2003; **44** (6): 1305-10.
4. Anderson DF, Prabhasawat P, Alfonso E, *et al*. Amniotic membrane transplantation after the primary surgical management of band keratopathy. *Cornea* 2001; **20** (4): 354-61.
5. Espana EM, Crueterich M, Sandoval H, *et al*. Amniotic membrane transplantation for bullous keratopathy in eyes with poor visual potential. *J Cataract Refract Surg* 2003; **29** (2): 279-84.
6. Tang GG, Ahn HB, Park WC. The clinical effects of dye-amniotic membrane transplantation on bullous keratopathy. *J Korean Ophthalmol Soc* 2003; **44** (8): 1741-7.
7. Choi YS, Kim JY, Wee WR, *et al*. Effect of application of human amniotic membrane on rabbit corneal wound healing after excimer laser photorefractive keratectomy. *Cornea* 1998; **17** (4): 389-95.
8. Woo HM, Kim HS, Kweon OK, *et al*. Effects of amniotic membrane on epithelial wound healing and stromal remodeling after excimer laser keratectomy in rabbit cornea. *Br J Ophthalmol* 2001; **85** (3): 345-9.
9. Paridaens D, Beekhuis H, van Den Bosch W, *et al*. Amniotic membrane transplantation in the management of conjunctival malignant melanoma and primary acquired melanosis with atypia. *Br J Ophthalmol* 2001; **85** (6): 658-61.
10. Espana EM, Prabhasawat P, Grueterich M, *et al*. Amniotic membrane transplantation for reconstruction after excision of large ocular surface neoplasias. *Br J Ophthalmol* 2002; **86** (6): 640-5.
11. Ti SE, Tseng SC. Management of primary and recurrent pterygium using amniotic membrane

transplantation. *Curr Opin Ophthalmol* 2002; **13** (4): 204-12.

12. Shimazaki J, Kosaka K, Shimmura S, *et al*. Amniotic membrane transplantation with conjunctival autograft for recurrent pterygium. *Ophthalmology* 2003; **110** (1): 119-24.
13. Tseng SC, Prabhasawat P, Lee SH. Amniotic membrane transplantation for conjunctival surface reconstruction. *Am J Ophthalmol* 1997; **124** (6): 765-74.
14. Jain S, Rastogi A. Evaluation of the outcome of amniotic membrane transplantation for ocular surface reconstruction in symblepharon. *Eye* 2004; **18** (12): 1251-7.
15. Tamhane A, Vajpayee RB, Biswas NR, *et al*. Evaluation of amniotic membrane transplantation as an adjunct to medical therapy as compared with medical therapy alone in acute ocular burns. *Ophthalmology* 2005; **112** (11): 1963-9.
16. Gomes JA, dos Santos MS, Cunha MC, *et al*. Amniotic membrane transplantation for partial and total limbal stem cell deficiency secondary to chemical burn. *Ophthalmology* 2003; **110** (3): 466-73.
17. Tseng SC, Prabhasawat P, Barton K, *et al*. Amniotic membrane transplantation with or without limbal allograft for corneal surface reconstruction in patients with limbal stem cell deficiency. *Arch Ophthalmol* 1998; **116** (4): 431-41.
18. Uçakhan OO, Köklü G, Firat E. Nonpreserved human amniotic membrane transplantation in acute and chronic chemical eye injuries. *Cornea* 2002; **21** (2): 169-72.
19. Nubile M, Carpineto P, Lanzini M, *et al*. Multilayer amniotic membrane transplantation for bacterial keratitis with corneal perforation after hyperopic photorefractive keratectomy: case report and literature review. *J Cataract Refract Surg* 2007; **33** (9): 1636-40.
20. Gicquel JJ, Bejjani RA, Ellies P, *et al*. Amniotic membrane transplantation in severe bacterial keratitis. *Cornea* 2007; **26** (1): 27-33.
21. Kim JS, Kim JC, Hahn TW, *et al*. Amniotic membrane transplantation in infectious corneal ulcer. *Cornea* 2001; **20** (7): 720-6.
22. Solomon A, Meller D, Prabhasawat P, *et al*. Amniotic membrane grafts for non traumatic corneal perforations, descemetoceles, and deep ulcers. *Ophthalmology* 2002; **109** (4): 694-703.
23. Ma DH, Wang SF, Su WY, *et al*. Amniotic membrane graft for the management of scleral melting and corneal perforation in recalcitrant infectious scleral and corneoscleral ulcers. *Cornea* 2002; **21** (3): 275-283.
24. Hosseini H, Nejabat M, Mehryar M, *et al*. Bevacizumab inhibits corneal neovascularization in an alkali burn induced model of corneal angiogenesis. *Clin Exp Ophthalmol* 2007; **35** (8): 745-8.
25. Lee SH, Tseng SC. Amniotic membrane transplantation for persistent epithelial defects with ulceration. *Am J Ophthalmol* 1997; **123** (3): 303-12.
26. Dua HS, Gomes JAP, King AJ, *et al*. The amniotic membrane in ophthalmology. *Surv Ophthalmol* 2004; **49** (1): 51-77.
27. Kim JC, Tseng SC. Transplantation of preserved human amniotic membrane for surface reconstruction in severely damaged rabbit corneas. *Cornea* 1995; **14** (5): 473-84.
28. Meller D, Pires RT, Mack RJ, *et al*. Amniotic membrane transplantation for acute chemical or thermal burns. *Ophthalmology* 2000; **107** (5): 980-9.
29. Jeoung JW, Yoon YM, Lee JL, *et al*. The effect of amniotic membrane transplantation on the treatment of necrotizing scleritis after pterygium excision. *J Korean Ophthalmol Soc* 2004; **45** (12): 1981-8.
30. Fernandes M, Sridhar MS, Sangwan VS, *et al*. Amniotic membrane transplantation for ocular

surface reconstruction. *Cornea* 2005; **24** (6): 643-53.

31. Sangwan VS, Burman S, Tejwani S, *et al*. Amniotic membrane transplantation: a review of current indications in the management of ophthalmic disorders. *Indian J Ophthalmol* 2007; **55** (4): 251-60.
32. Niknejad H, Peirovi H, Jorjani M, *et al*. Properties of the amniotic membrane for potential use in tissue engineering. *Eur Cell Mater* 2008; **15**: 88-99.
33. King AE, Paltoo A, Kelly RW, *et al*. Expression of natural antimicrobials by human placenta and fetal membranes. *Placenta* 2007; **28** (2-3): 161-9.
34. Ni J, Abrahamson M, Zhang M, *et al*. Cystatin E is a novel human cysteine proteinase inhibitor with structural resemblance to family Z cystatins. *J Biol Chem* 1997; **272** (16): 10853-8.
35. Kamal JD, Pfaller MA, Rempe LE, *et al*. Reduced intravascular catheter infection by antibiotic bonding: a prospective, randomized, controlled trial. *JAMA* 1991; **265** (18): 2364-8.
36. Raad I, Darouiche R, Hachem R, *et al*. Antibiotics and prevention of microbial colonization of catheters. *Antimicrob Agents Chemother* 1995; **39** (11): 2397-400.
37. Alonso-Echanove J, Edwards JR, Richards MJ, *et al*. Effect of nurse staffing and antimicrobial-impregnated central venous catheters on the risk for bloodstream infections in intensive care units. *Infect Control Hosp Epidemiol* 2003; **24** (12): 916-25.
38. Eveillard M, Mertl P, Tramier B, *et al*. Effectiveness of gentamicin-impregnated cement in the prevention of deep wound infection after primary total knee arthroplasty. *Infect Control Hosp Epidemiol* 2003; **24** (10): 778-80.