Amniotic Membrane Extract for Acute Ocular Chemical Burns

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Abstract: Background: Ocular chemical burns induce devastating and permanent damage to the ocular surface. Rapid intervention is required for maximal visual rehabilitation. Amniotic membrane transplantation (AMT) may save the ocular surface, however it introduces a potentially unnecessary surgical trauma in such compromised eyes. Amniotic membrane extracts (AME) could be a practical substitute of AMT in acute chemical burn. Aim: To evaluate the efficacy of topical AME in the management of acute ocular chemical burn. Methods: Non-comparative interventional case series. Six eyes of 4 consecutive patients with mild to moderate acute chemical burn, exhibiting persistent epithelial defect, inflammation and haze despite extensive conventional therapy were recruited. Topical AME was prepared and added to the conventional treatment within 2 days of the injury. Pain relief, inflammation, haze, and corneal epithelial healing were monitored. Results: Pain was significantly relieved, and inflammation was markedly reduced in all cases. The corneal epithelial defects rapidly healed while visual acuity improved within 11 (range 4-23) days. During an average follow-up period of 6 months (range, 3-8 months), all eyes retained stable surface with improved corneal clarity without neovascularization or symblepharon. Conclusions: Topical application of AME could be an effective adjunct in the treatment of mild to moderate cases of acute chemical burns. It allows non-traumatic and economic early intervention to promote epithelialization, reduce pain, haze and inflammation in acute phase, and prevent cicatricial complications in chronic phase. This result justifies additional large series controlled studies in the future.

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Key words: Acute chemical burn, amniotic membrane extract, corneal epithelial defect

1. Introduction:

Ocular chemical burns injury is a serious ocular emergency in which rapid, devastating, and permanent damage can occur. The severity of the injury correlates directly to exposure duration and the causative agent. Treatment of such injuries requires medical and surgical intervention, both acutely and in the long term. Regardless of the underlying chemical involved, the common goals of management include removing the offending agent, controlling inflammation and promoting ocular surface healing with maximal visual rehabilitation. [1]

Various medical therapies have been used to achieve these objectives, including topical and systemic ascorbate, citrate, tetracycline, progesterone and steroids.[2-7] Previous studies revealed that early intervention with amniotic membrane transplantation (AMT) in mild and moderate chemical burns results in a marked reduction of symptoms, rapid restoration of the ocular surface, and improved visual acuities while preventing cicatricial complications in the chronic stage. [8-17]. However, surgically performed AMT renders a relatively high cost and potentially unnecessary surgical trauma in such compromised eyes. Furthermore, the membrane patch usually dissolves within several days so that multiple sessions of AMT may be required. [8; 16; 17] Recent studies have shown that topical amniotic membrane extracts (AME) has a comparable effect to AMT in promoting epithelialization, decreasing inflammation, and suppressing corneal neovascularization.[18-21] Therefore, we hypothesized that AME could be a practical substitute for AMT in acute chemical burns. Herein we reported our experience in preparing and using AME as a rapid, economic, non-traumatic alternative modality in the treatment of acute chemical burns.

2. Methods

Patients:

This study was conducted, according to the tenets of the Declaration of Helsinki, to evaluate the effect of AME as an adjunct in the treatment of mild to moderate acute ocular chemical burns. This small series included 6 eyes of 4 patients, all males, with a mean age of 34.5 ± 15.8 (range, 19-56 years). Their demographic data and clinical characteristics are summarized in Table 1. After obtaining a written informed consent, all patients received topical AME in combination with conventional treatment within 2 days following the onset of chemical burn. The injury was bilateral in 2 patients (Cases 1 and 2) and unilateral in the other two (Cases 3 and 4). The causative agents were alkali in 3 patients and acidic substance in one (Case 4).

Upon presentation, all patients complained of significant ocular pain, light sensitivity and blurred vision. Corneal epithelial defects were partial [Cases 1, 2 and 4 (Fig 1A, 1B)] or total [Cases 2 and 3 (Fig 2A, 2B)] and with various degrees of limbal and

conjunctival involvement. In addition, 4 eyes had limbal ischemia ranging from 2 to 6 clock hours. The severity was classified as Grade, I (2 eye), Grade II (2 eyes) and Grade III (2 eyes) based on the criteria defined by Roper-Hall.[22] Microorganism culture of all ulcer smears was negative and intraocular pressure (IOP) was normal in all cases.

All patients were initially treated with conventional medical therapies including saline/water irrigation to normalize the ocular surface pH, removal of remaining particulate materials, topical antibiotics, lubricants, 10% ascorbic acid and 6% citrate, antibiotic-steroids and cycloplegics, oral vitamin C or a combination thereof for the first 2 days after injury. When improvement was not apparent, all patients were detailed with information about the clinical course of chemical burns of the eye, alternative treatments, and advantages and disadvantages of AME and AMT. After a written consent was obtained, AME eye drops were added to the regimen.

Preparation of AM and extraction was carried out under sterile conditions. Each placenta was rinsed with sterile saline solution containing 1% penicillin-streptomycin-neomycin (PSN) antibiotic mixture (Invitrogen/Gibco, Grand Island,NY). After peeling off from the attached chorion, AM was submerged in liquid nitrogen. Under cold conditions, the membrane was cut into small pieces, manually ground into fine powder, and homogenized with normal saline. The homogenate was then centrifuged twice at 15,000 rpm for 30 minutes at 4°C. The supernatant was collected and the protein concentration was measured by DC protein assay (Bio-Rad, Hercules, CA). Non preserved 100μg/ml eye drops were prepared and kept frozen at -20°C.

AME was instilled hourly for the first week, once every two hours until complete re-epithelialization were achieved, then 4 times daily for 2 weeks and was tapered off thereafter.

Subjective symptoms were scaled at each visit as 0 (No discomfort, No haze), 1 (Mild discomfort and/or Mild haze), 2 (Moderate discomfort and/or Moderate haze) and 3 (Severe discomfort and/or Dense haze). Ocular surface inflammation was graded as 0 (absent), 1 (mild), 2 (moderate), 3 (severe). Fluorescein staining was conducted to evaluate epithelialization.

3. Results:

The average follow-up period was 6±2.3 months (range, 3-8 months). The results were summarized in Table 1. All patients reported a significant relief of pain and light sensitivity within the first week after AME treatment with overall symptomatic scores reduced from 2-3 to 0-1. Inflammation scores significantly decreased from 2-3 at first visit to 0-1 at the second week.

Rapid and progressive epithelialization was also observed in all eyes depending on the severity; for Grade I injury (Case 4, Fig 1) with less limbal involvement the epithelialization was completed in a centripetal manner. However, for Grade III injuries (Cases 3, Fig 2) with extensive limbal involvement the epithelialization started circumferentially to close the limbal defect before moving centripetally to close the corneal defect. After AME treatment, complete epithelization was obtained in all eyes within a mean period of 11 days (range, 4-23 days, Table 1).

Accompanied with re-epithelialization, corneas, which were initially presented with edema and haze became clear (Fig 1E), or left with mild haze without edema (Fig 2E).

Impression cytology analysis was then performed after the ocular surface had been stable for more than three months, where no limbal stem cell deficiency was noted. There were no cicatricial complications such as symblepharon at the final visit (Fig 1F, 2F). Best corrected visual acuities (BCVA) improved to 20/20 in 5 eyes (83%) while Case 3 had BCVA of 20/60 due to residual corneal haze (Table 1). The ocular surface remained stable in all eyes during the follow up period.

Table 1. Results of AME in treating acute chemical burns.

Case No.	Eye No.	Agent	Grade	Limbal Ischemia (CH)	Symptoms Score		BCVA		Inflammation Score		Epithelialization (Days)
					Before	After	Before	After	Before	After	
1	1	Alkali	I	0	2	0	20/40	20/20	2	0	6
	2		II	2	3	0	20/50	20/20	3	0	8
2	3	Alkali	III	6	3	1	20/60	20/20	3	0	21
	4		II	4	2	0	20/400	20/20	3	1	12
3	5	Alkali	III	6	3	1	20/400	20/60	3	1	15
4	6	Acid	I	0	2	0	20/200	20/20	2	0	4

Note: CH: Clock Hours, BCVA: best corrected visual acuity

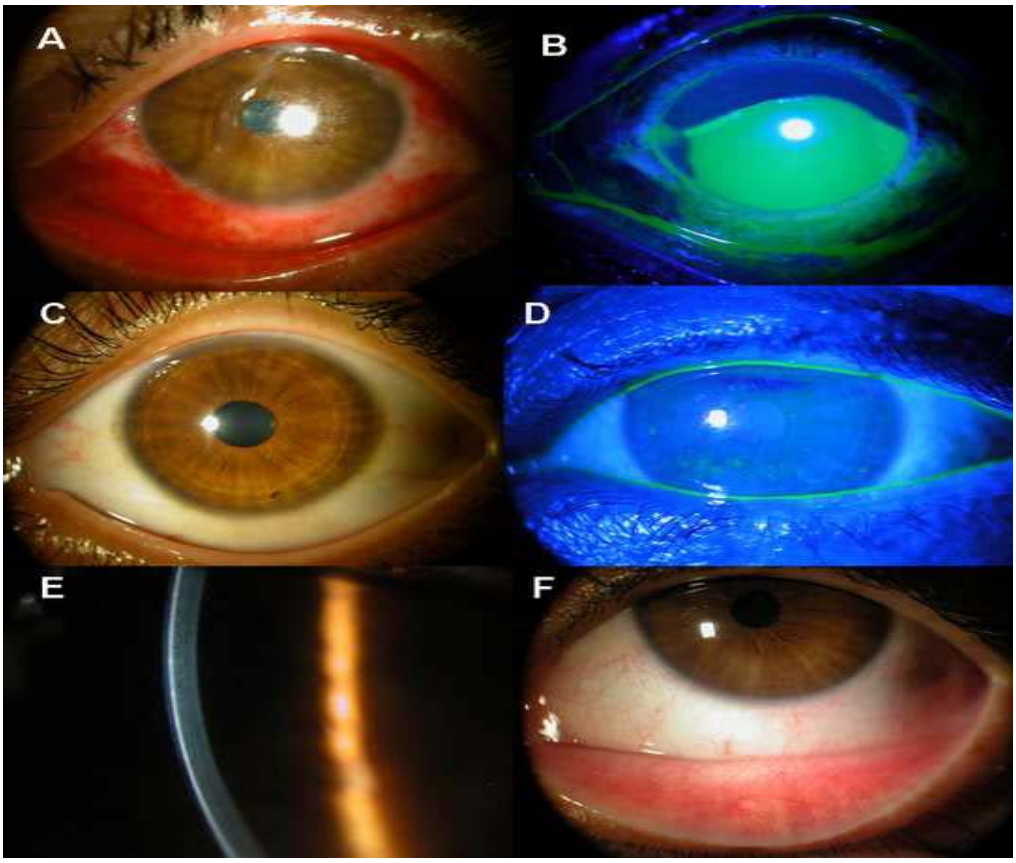

Fig. 1

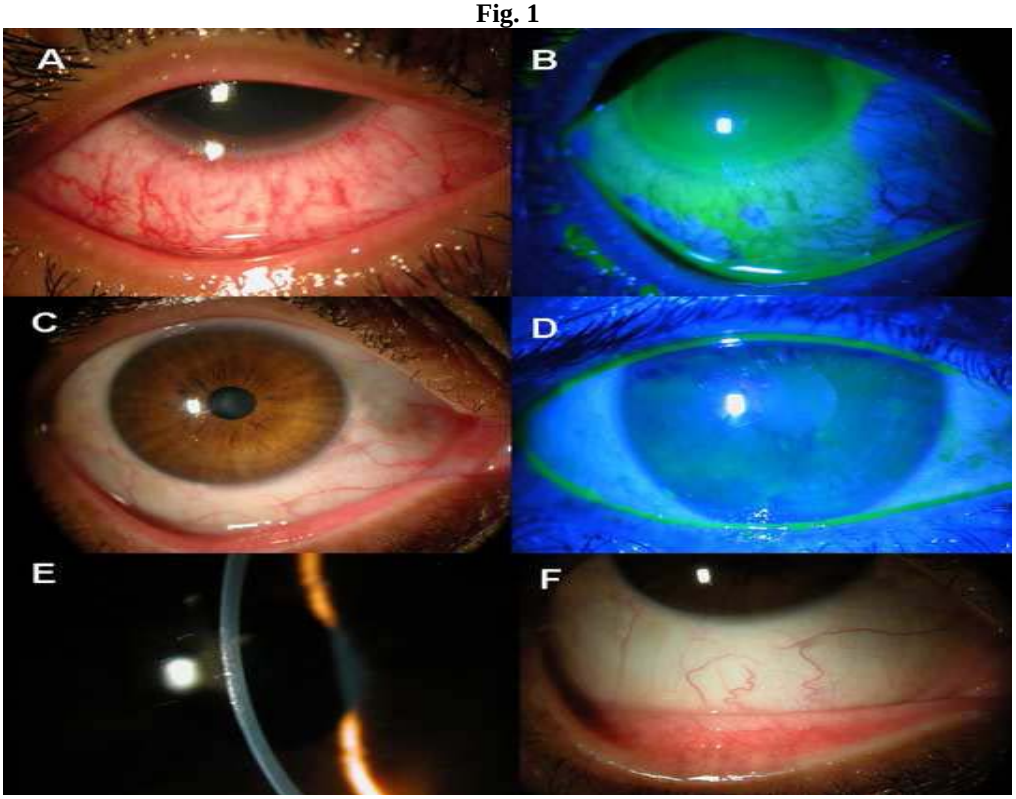

Fig. 2

4. Discussion:

Inflammatory mediators released from the ocular surface at the time of chemical burns induce tissue necrosis and attract further inflammatory reaction. This vigorous inflammatory response not only inhibits re-epithelialization but also increases the risk of corneal ulceration and perforation. In this non-comparative case series, AME was prepared and applied simultaneously with the conventional treatment to help break this inflammatory cycle and promote healing in patients with acute chemical burn with and without limbal involvement. The results of the present study revealed that early application of AME could be effective in rapidly relieving symptoms, reducing inflammation, and promoting epithelialization in mild to moderate acute chemical burn and consequently. It may also thwart limbal stem cell deficiency (LSCD) and symblepharon at the chronic stage.

Human amniotic membrane transplantation has been widely performed as a therapy for a variety of ocular surface disorders, and has been known to be highly effective not only in promoting re-epithelialization but also in suppression of inflammation. The mechanisms of action of the amniotic membrane transplantation are considered to include prolongation and clonogenic maintenance of epithelial progenitor cells, promotion of goblet and non-goblet cell differentiation, suppression of Transforming Growth Factor beta signaling, myoblastic differentiation of normal fibroblasts, and exclusion of inflammatory cells.[23] Based on the inherent biological actions known to amniotic membrane,[24-26] several investigators have explored the clinical efficacy of deploying AMT as a temporary graft to reduce inflammation and to promote healing in acute chemical burns.[9;12-14;17] Application of AME as eye drops is a different approach for the treatment of alkali injures; Bonci et al [21] prepared a suspension containing homogenized amniotic membrane to investigate its beneficial effect on ocular surface diseases. They used this suspension to treat 21 patients with different ocular diseases exhibiting inflammation and epithelial defects; re-epithelialization was completed after 15–30 days with no side effects. However, they focused on the beneficial effects of the amniotic stromal matrix, rather than on the function of viable amniotic cells. It has been reported that amniotic cells have multiple functions, such as the synthesis and release of neurotransmitters [27-32] and neurotrophic factors [33; 34] and are a source of soluble anti-inflammatory factors. [20] In our study we modified the technique described by Jiang et al, [18] to ensure keeping all the active ingredients of AM by avoiding heat and filtration.

Despite variable extents of ocular surface epithelial defects, all patients reported significant relief of pain and light sensitivity within the first week after AME treatment.

Although one may attribute AME's effect in relieving pain to its anti-inflammatory action, we suspect that such a rapid action in pain relief might be mediated by a yet unknown anti-pain action that deserves further investigation.

Ocular surface inflammation was markedly reduced in all cases after treatment. Although the exact action mechanism remains to be determined, the aforementioned effect may be associated with early delivery of AM's anti-inflammatory active ingredients, which are retained in AME.[18-21] This anti-inflammatory effect is crucial in the treatment of chemical burns, as inflammation severely threatens stem cell survival[35], aggravates neovascularization, and induces apoptosis of keratocytes and stromal melting[36]. While topical corticosteroids in chemical burn are debatable, AME can function as an inflammation inhibitor without side effects.

When AM was used as a temporary patch, polymorphonuclear cells rapidly adhere to its stromal side in rabbit models of alkali burns [10] and in human patients with chemical burns, [37; 38] where these adherent cells underwent rapid apoptosis. [38, 39] Similarly, mononuclear cells, including lymphocytes and macrophages also underwent rapid apoptosis when adherent onto AM stroma in a murine model of HSV-induced necrotizing stromal keratitis. [40; 41] Such a unique anti-inflammatory action of the AM by promoting cellular apoptosis has been recapitulated in an in vitro culturing system using murine macrophages, [42] and recently He et al, reported that such an activity could be retained in AME. [43]

Although AME lacked the bandage effect of AMT, epithelialization started as early as four days in cases with nearly intact limbus; however, it took longer when there was nearly total limbal involvement with/without regional ischemia. It has to be noted that no impression cytology was performed at the initial visits, and limbal stem cell dysfunction was assumed clinically in cases with limbal ischemia. All eyes maintained corneal integrity without stromal melting. We speculate AME might be responsible for inhibiting such a stromal melting through multiple mechanisms: *first,* by maintaining the balance between the matrix metalloproteinase (MMP) and tissue inhibitor of matrix metalloproteinase (TIMP),[44] as TIMPs were found in epithelial and mesenchymal cells as well as in the compact layer of the amniotic membrane stroma, [45] and *second,* through reducing keratocyte apoptosis or modifying

the proliferation and migration of stromal fibroblasts.[39;46]

Interestingly, there was no abnormal limbal or peripheral corneal vascularization during the follow-up period. We further observed that the healed surface did not show any evidence of conjunctivalization, clinically or by impression cytology. We believe that limbal healing resulted from rapid recovery of limbal epithelial stem cells after the acute insult. Further investigation is needed to confirm the ability of AME to resurrect and promote in vivo expansion of limbal stem cells.

Collectively, our results showed that AME could be an effective adjunct in the treatment of mild and moderate cases of acute chemical burn by promoting healing, reducing inflammation, and restoring vision. Although we noted that instillation of AME in combination with the conventional therapy could successfully deliver AM's desirable actions, our series was still too small to determine its efficacy in managing different types of chemical burns. Additional controlled studies are needed to confirm that AME is a safe, non-traumatic, convenient, and economical alternative therapy to enhance corneal wound healing in acute chemical burn as well as other inflammatory ocular surface diseases.

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