Amniotic Membrane Extract Eye Drop Promotes Limbal Stem Cell Proliferation and Corneal Epithelium Healing

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Abstract

Objective: Human amniotic membrane (HAM) is used as a supporter for limbal stem cell (LSC) expansion and corneal surgery. The aim of study is to use HAM extracts from healthy donors to enhance proliferation of LSCs in vitro and in vivo.

Materials and Methods: In this interventional experimental study, the effective and cytotoxic doses of the amniotic membrane extract eye drops (AMEED) was assessed by using different concentrations of AMEED (0-2.0 mg/ml) to LSC cultures for 14 days. Subsequently, the expression levels of ATP-binding cassette sub-family G member 2 (ABCG2, a putative stem cell marker), cytokeratin 3 (K3, corneal marker), K12 and K19 (corneal-conjunctival cell markers) were assessed by real-time polymerase chain reaction (PCR). In the second step, the corneal epithelium of 10 rabbits was mechanically removed, and the right eye of each rabbit was treated with 1 mg/ml AMEED every 2 hours (group 1) or every 6 hours (group 2). The left eyes only received an antibiotic. The corneal healing process, conjunctival infection, degree of eyelid oedema, degree of photophobia, and discharge scores were evaluated during daily assessments. Finally, corneal tissues were biopsied for pathologic evidences.

Results: In comparison to the positive control [10% foetal bovine serum (FBS)], 0.1-1 mg/ml AMEED induced LSC proliferation, upregulated ABCG2, and downregulated K3. There were no remarkable differences in the expression levels of K12 and K19 (P<0.05). Interestingly, in the rabbits treated with AMEED, the epithelium healing duration decreased from 4 days in the control group to 3 days in the two AMEED groups, with lower mean degrees of eyelid oedema, chemosis, and infection compared to the control group. No pathologic abnormalities were observed in either of the AMEED groups.

Conclusion: AMEED increases LSCs proliferation ex vivo and accelerates corneal epithelium healing in vivo without any adverse effects. It could be used as a supplement for LSC expansion in cell therapy.

Keywords: Amniotic, Corneal Healing, Proliferation, Stem Cell


Introduction

For the past 75 years, human amniotic membrane (HAM) has been used in ocular surgery and as a supporter for limbal stem cell (LSC) expansion (1). The anti-inflammatory, anti-scarring, anti-microbial, anti-angiogenic, anti-fibrotic effects, and low immunogenicity of HAM (1, 2) make it suitable for surgical applications without necessitating the use for systemic immunosuppressive drugs. HAMs produce growth factors that can promote re-epithelialization of the cornea, including epidermal growth factor (EGF), keratinocyte growth factor (KGF), vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) (2, 3). The presence of structural proteins such as collagen (I, III, VI, and VII), laminin, fibronectin, lumican, and osteoglycin can help to explain the observed epitheliotrophic effects of HAM (2-4). Thus, the specific structure of HAM leads to the promotion of re-epithelialization and other aspects of the corneal healing process. In particular, HAM induces LSC migration, inhibits apoptosis, and maintains epithelial progenitor cells within the LSC niche (5, 6).

Advances in stem cell research that include the development of laboratory techniques for isolation and maintenance of LSCs and improvements in surgical techniques have opened a new chapter for the application of bioengineered grafts by enabling ex vivo LSC expansion. HAM (either intact or denuded) was the first tissue used as a carrier for ex vivo LSC expansion (7, 8). However, the potential disadvantages of amniotic membrane transplantation include donor variation (9), increased risk of viral infections due to the use of fresh tissue, difficulties in HAM manipulation, increased surgery time, and increased risk of complications such as granuloma formation, giant papillary conjunctivitis, and patient discomfort (10).

In recent years, several studies have researched the