Journal français d'ophtalmologie (2014) 37, 358—364

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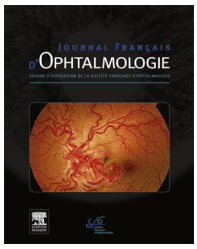

ORIGINAL ARTICLE

Subconjunctival amniotic membrane free graft in rabbit eyes: Effects on fibrovascular reaction

Transplantation d'une membrane amniotique libre sous la conjonctive chez les lapins : effets sur la réaction fibrovasculaire

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Received 15 May 2013; accepted 25 September 2013
Available online 3 April 2014

KEYWORDS
Amniotic membrane;
Subconjunctival amniotic membrane;
Subconjunctival fibrovascular reaction;
Amniotic membrane transplantation

Summary
Purpose. — The purpose of our study is to investigate the effect of subconjunctival amniotic membrane free graft on subconjunctival fibrovascular reaction.
Methods. — Twelve healthy male white New Zealand rabbits were used for the study. The rabbits were divided randomly into two groups: Study Group ($n=6$) and Control Group ($n=6$). In the Study Group, a 4 mm limbal incision was made and a 4×4 mm subconjunctival pocket was created with blunt dissection. A 4×4 mm single layer of free amniotic membrane was placed in the pocket in an epithelium-up fashion without suturing. The limbal opening was secured with 10-0 nylon sutures on both sides. In the Control Group, a 4 mm limbal incision was made, a 4×4 mm subconjunctival pocket was created with blunt dissection, and the limbal opening was closed with 10-0 nylon sutures on both sides. After the first month, sclero-conjunctival blocks were obtained from the operated area and sections were stained with hematoxylin and eosin, Masson trichrome, and Ki67, SMA and CD34 antibodies.
Results. — The number of fibroblasts, lymphocytes and macrophages was significantly higher in the Study Group than in the Control Group. The number of Ki67- and SMA-positive cells, and CD34-positive vessels was also significantly higher in the Study Group. Amniotic membrane appeared to form folds in all the specimens.

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http://dx.doi.org/10.1016/j.jfo.2013.09.011

Conclusion. — The higher fibrovascular reaction shown by our histopathological examination indicates that free human amniotic membrane grafting without suturing is not useful in decreasing the subconjunctival fibrovascular reaction at the first postoperative month in rabbit eyes.

MOTS CLÉS
Membrane amniotique ;
Membrane amniotique sous la conjonctive ;
La réaction fibrovasculaire sous-conjonctivale ;
Transplantation de membrane amniotique

Résumé
Objectif. — Notre étude avait pour but d'examiner l'effet sur la réaction fibrovasculaire d'une transplantation de membrane amniotique libre sous la conjonctive.
Méthode. — Nous avons utilisé douze lapins blancs de Nouvelle-Zélande mâles et en bonne santé. Les lapins ont été répartis en deux groupes au hasard : le groupe à l'étude ($n = 6$) et le groupe témoin ($n = 6$). Dans le groupe à l'étude, on a effectué une incision limbique de 4 mm et créé une poche sous-conjonctivale de 4×4 mm à l'aide d'une dissection mousse. Une monocouche de membrane amniotique libre de 4×4 mm a été placée dans la poche sans effectuer de sutures de manière à ce que le haut de l'épithélium soit en position supérieure. L'ouverture limbique a été attachée des deux côtés à l'aide de sutures en nylon 10-0. Dans le groupe témoin, on a effectué une incision limbique de 4 mm et créé une poche sous-conjonctivale de 4×4 mm à l'aide d'une dissection mousse. Puis, l'ouverture limbique a été refermée des deux côtés à l'aide de sutures en nylon 10-0. À la fin du premier mois, on a obtenu des blocs scléroconjonctivaux à partir de la zone ayant subie l'opération puis on a effectué des coupes qui ont été colorées à l'hématoxyline, à l'éosine, au trichrome de Masson ainsi qu'aux anticorps Ki67, SMA et CD34.
Résultats. — Le nombre de fibroblastes, de lymphocytes et de macrophages était significativement supérieur dans le groupe à l'étude que dans le groupe témoin. De plus, le nombre de cellules positives au Ki67 et au SMA ainsi que le nombre de vaisseaux positifs au CD34 étaient significativement supérieurs dans le groupe à l'étude. La membrane amniotique semblait former des plis chez tous les spécimens.
Conclusions. — La réaction fibrovasculaire supérieure démontrée par notre examen histopathologique indique que la transplantation d'une membrane amniotique libre sans sutures n'est pas utile pour diminuer la réaction fibrovasculaire sous-conjonctivale chez les lapins un mois après l'opération.

Introduction

Any surgical intervention on the conjunctiva activates the wound-healing cascade in which fibroblasts play a significant role. Excessive fibrosis and scarring after trabeculectomy and glaucoma drainage device implantation leads to decreased success rates and elevated intraocular pressure which needs further medical or surgical intervention. Similarly, excessive scarring especially after repeated strabismus surgery is a major complication and compromises the effect of surgery [1]. To prevent these complications, mitomycin C and 5-fluorouracil have been widely used in glaucoma surgery [2,3].

Amniotic membrane (AM) modulates the multi-step healing and fibrosis cascade which includes the release of blood cells and plasma proteins into the damaged site; the activation of clotting and complement systems; release of growth factors; neutrophil, macrophage and lymphocyte migration to the wound site following by the fibroblast migration and proliferation and eventually wound contraction [4—6]. After the identification that the stromal matrix of AM suppresses DNA synthesis and subsequent differentiation of fibroblasts through suppressing the TGF-β signaling system [7,8], which is the most powerful stimulant of proliferation, migration and collagen synthesis of human Tenon's fibroblasts [4,7—10], the AM has been also evaluated for the purpose of inhibiting postoperative fibroblastic proliferation. The anti-angiogenic, anti-fibrotic and anti-inflammatory effects of AM have been related to the action of covalent complex of hyaluronan and the heavy chain of inter-α-inhibitor [5,6].

The serious complications, which can occur with mitomycin C and 5-fluorouracil, when used to inhibit the excessive fibrovascular reaction after glaucoma and pterygium surgery, have created the need for safer options such as amniotic membrane [2,3].

The rational of this study was based on the immunologically inert nature, anti-angiogenic [6] and anti-fibrotic properties of AM [7,8]. The outcomes of clinical studies in which AM was used to prevent excessive wound healing and unwanted fibrosis in pterygium, glaucoma and strabismus surgeries were the additional starting point of this study [11—21]. The purpose of our study is to investigate the effect of subconjunctivally transplanted free amniotic membrane on subconjunctival fibrovascular reaction.

Methods

This experimental study was approved by the Local Ethics Committee of Animal Experiments (Ankara Training and Research Hospital, Ankara, Turkey), and conducted in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Visual Research. The study was conducted in