中文計劃名稱

角膜間質層角質細胞的自殺行為與角膜移植手術的關係

英文計劃名稱

The association of keratocytic apoptosis with corneal transplantation

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關鍵詞

角膜，自殺反應，角膜間質細胞，角膜表皮細胞，全層角膜移植

Key Words

cornea, keratocyte, apoptosis, penetrating keratoplasty, corneal epithelial cell

中文摘要

角膜細胞自殺反應為近幾年來國內外文獻的熱門研究主題。無論在準分子雷射近視手術或角膜移植手術當中，角膜細胞的反應都將可能影響術後的傷口復原或視力進步狀況。本計劃乃利用紐西蘭白兔的角膜作為研究材料。旨在探究不同的角膜貯存方式，或角膜運送過程中的晃動現象是否引發角質細胞自殺反應；而本實驗中有關角質細胞自殺反應的強度，乃以 TUNEL Assay **偵測。由本實驗結果初步得知，角膜保存時間愈久，處理過程中經過搖晃等等將導致較嚴重角膜表皮細胞或角膜間質細胞的自殺行為。**

Abstract:

Several investigators have shown recently that anterior stromal keratocytes undergo programmed cell death (apoptosis) in response to corneal epithelial injury, and it is believed that this phenomenon plays an important role in wound healing after many kinds of corneal surgery such as photorefractive keratectomy (PRK). However, It is not known whether degree

of apoptosis may account for variations of wound healing observed after penetrating keratoplasty (PKP). The purpose of this study is to investigate whether keratocyte apoptosis takes place during storage and process period of donor cornea. New Zealand white rabbits are used as the animal models. The result of this study showed that the way of preservation and transportation will affect keratocyte apoptosis in corneal buttons.

計劃緣由及目的

The finding of keratocyte apoptosis

In the past, the stromal keratocytes of adult cornea have been characterized as quiescent cells populating the stroma. Recent researches and the introduction of laser refractive procedures have made this notion more important.
Keratocyte apoptosis extend to a depth of 50to 200 micrometer, depending on the magnitude of the epithelial injury and the species studied. Typical in situ staining with the terminal deoxyribonucletidyl transferase-mediated d-uridine 5'-triphosphate-dihoxigenin nick-end labeling (TUNEL) assay is first noted at approximately 1 hour after injury, but it has been noted to peak at approximately 4 hours later .The later prominence of TUNEL staining likely is caused by continued internucleosomal deoxyribonucleic acid (DNA) fragment after the earliest cell morphologic changes are noted. Control of this response has the potential to regulate wound healing in the cornea in response of corneal surgeries, such as photorefractive keratectomy (PRK), laser-assisted in situ keratomileusis (LASIK) or penetrating keratoplasty (PKP).

Keratocyte apoptosis as an initiator of the wound-healing response in the cornea

Cellular morphologic changes consistent with apoptosis are observable in keratocytes immediately after an injury to the corneal epithelium. The disappearance of the keratocytes is noted after the scraping of the epithelium in preparation for excimer laser photorefractive keratectomy (PRK), after a microkeratome injury to the epithelium in laser-assisted in situ keratomileusis (LASIK) , and is in association with manipulations that occur during other corneal surgical procedures. The keratocytes that die are thought to be replaced over a period of time of a few days by proliferation and migration of remaining keratocytes from the posterior and peripheral stroma. The activated keratocytes that replenished the anterior stroma, however, produce increases amounts of disorganized collagen and other components associated with stromal healing and regression of the effect of PRK).The activated keraticytes also express increased hepatocyte growth factor and other growth factors, which may stimulate proliferation and inhibit terminal differetiation of corneal epithelial cells.

Effect of storage condition on corneal epithelial preservation

The success of penetrating keratoplasty depends on many perioperative factors, including preoperative, operative and postoperative management of recipients and donors. However, the epithelial condition of the donor buttons has long been ignored. It may not be a major problem in western countries with the new corneal preservative media; however, it remained a major concern in many other areas of the world. The period prior to arrival at the

donor button's destination is long, and there is unavoidable disturbance during transportation. Is there any effect on the epithelium of corneal buttons from long-term vibration? Does the macroscopic or microscopic epithelial defects trigger keratocytic apoptosis of corneal buttons like cornea in vivo? Is there any possibility to inhibit this phenomenon during the storage period of donor button? We want to resolve this questions in this study.

Delayed post-operative epithelial healing after penetrating keratoplasty

Penetrating keratoplasty has been recognized as a very effective surgical method to treat lots of corneal problems. But unfortunately, there still exists some problems to be solved. For example, persistent epithelail defect after operation may cause lots of postoperative problems such as corneal melting, calcium deposotion and infection, and bother many ophthalmologists.

Since keratocyte apoptosis was found to take place after corneal epithelial damage in vivo, and this is supposed to play some role in post-traumatic or post-operative wound healing, it is interesting to investigate the effect of keratocyte apoptosis of donor buttons on postoperative wound healing, and we further focus it on postoperative poor epithelization. What is the effect of this reaction on postoperative results? Which steps from donor cornea harvesting to transplantation may trigger keratocyte apopatosis? Can we block this phenomenon and improve the surgical results? All these questions remained to be solved.

結果與討論

Group 1. Keratocytic apoptosis directly after removing the corneal epithelium of the central cornea of 8 mm diameter

Cornea	**TUNEL-positive keratocytes (%)**			
	Under intact epithelium	**Under debrided epithelium**		
		Anterior Stroma	**Middle Stroma**	**Posterior stroma**
1	5 %	78%	24%	3%
2	10%	64%	12%	6%
3	6%	50%	16%	5%
4	8%	68%	8%	2%

Group 2 Donar cornea storaged in moist chamber at 4 °C.

Cornea	**Storage Time**	**Temperature**	**Epithelial condition**	**TUNEL-positive cells (%)**		
				Stroma	**Epithelium**	**Endothelium**
1	12 hr	4 °C.	Intact	0%	2%	0%
2	12 hr	4 °C.	Intact	0%	0%	2%
3	24 hr	4 °C.	Intact	0%	3%	1%

4	24 hr	4 °C.	Intact	1%	3%	0%
5	36 hr	4 °C.	Mildly exfoliated	3%	5%	4%
6	36 hr	4 °C.	Mildly Exfoliated	10%	5%	5%
7	48 hr	4 °C.	Totally exfoliated	36%	Totally exfoliated	8%
8	48 hr	4 °C.	Totally exfoliated	23%	Totally exfoliated	16%

Group 3. Donar cornea storaged in Optisol GS at 4 °C.

ornea	**Storage Time**	**Temperature**	**Epithelial condition**	**TUNEL-positive cells (%)**		
				Stroma	**Epithelium**	**Endothelium**
1	12 hr	4 °C	Intact	<5%	<5%	<5%
2	12 hr	4 °C	Intact	<5%	<5%	<5%
3	24 hr	4 °C	Intact	<5%	<5%	<5%
4	24 hr	4 °C	Intact	<5%	<5%	<5%
5	36 hr	4 °C	Intact	8%	6%	3%
6	36 hr	4 °C	Partial exfoliated	10%	4%	2%
7	48 hr	4 °C	Intact	5%	4%	5%
8	48 hr	4 °C	Partial exfoliated	22%	10%	12%

Group 4 Donar cornea put into Optisol GS at 4 °C. Shaking at a speed of 5 rpm (0.001475g) for 10 hours.

Cornea	**Storage Time**	**Temperature**	**Epithelial condition**	**TUNEL-positive cells (%)**		
				Stroma	**Epithelium**	**Endothelium**
1	12 hr	4 °C	Intact	<5%	<5%	<5%
2	12 hr	4 °C	Intact	<5%	<5%	<5%
3	24 hr	4 °C	Intact	<5%	<5%	<5%
4	24 hr	4 °C	Intact	<5%	<5%	<5%
5	36 hr	4 °C	Intact	8%	10%	5%

6	36 hr	4 °C	Partially exfoliated	15%	14%	12%
7	48 hr	4 °C	Partially exfoliated	18%	16%	16%
8	48 hr	4 °C	Partially exfoliated	25%	19%	16%

Figure 4 ***Cornea 7 in Group III***
After storage at Optisol GS at 4°C and shaked at 10 Hz for 48 hrs, the corneal epithelium was partially desquamated. TUNEL stain revealed the positive reaction at whole layers of keratocytes.

計劃結果自評

由本計劃初步結果得知，角膜間質細胞的自殺行為確與不同的角膜保存及處理過程有關。但本計劃結果的缺點是 TUNEL stain **的結果往往無法非常確定(因染色結果常常太淡);另外，隨著保存時的增長角膜間質細胞確實有較嚴重的自殺行為出現，但此表現卻不一定呈現線性關係，此乃為本實驗結果目前無法克服之處。**

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