·Basic Research·

Influence on cultured human keratocytes by liposome

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Abstract

• AIM: To observe the effects on human keratocytes by cationic liposome Lipofectamine™2000 (LF2000), to investigate the efficiency and safe range applied in human keratocytes, and establish basis for gene therapy of human keratocytes.

• METHODS: Human keratocytes cultured *in vivo* within 3 to 5 passages were used in experiment after being identified. The effects on proliferation of cultured human keratocytes by LF2000 with different concentrations and time were evaluated By MTT; the effects of LF2000 on the survival rate and its relation with 5,10,20,40,80mg/L concentration and time were detected by trypan blue staining.

• RESULTS: LF2000's effects on human keratocytes were related with concentration and time. The cellular proliferation and survival rate declined when concentration of LF2000 was above certain level, and this effect increased as time became longer. LF2000 had no effect with concentration under 40mg/L for 24 hours.

• CONCLUSION: LF2000 did not cause cytotoxicity during a concentration range "tested", and it is hoped to play an important role in gene therapy of human keratocytes.

• KEYWORDS: keratocytes; gene transfection; liposome

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INTRODUCTION

Changes of keratocytes in repair following trauma related closely with visual function. Clinicians have been looking for medicines which could modulate the repair of cornea trauma. Regulation on molecule and cell levels is hot at present. In this experiment, LF2000 of different concentration and time was used in cultured human keratocytes in order to obtain its safe range of concentration and time, also to provide experimental basis for gene prophylaxis and therapy of corneal diseases.

MATERIALS AND METHODS

Materials

Equipments Refrigerated centrifuge; attemperator; cellular incubator; inverted phase contrast microscope; brachytely homeothermia centrifuger (Hereus Company).

Reagent Lipofectamine™2000 (Gibco/BRL Company); DMEM nutritive medium (Gibco/BRL Company).

Methods

Primary culture and subculture of human keratocytes Cultured the corneal stroma tissues from donated healthy eyeball, and cells within 3-5 passages were used in the following experiment.

The effects on human keratocytes by Lipofectamine™ 2000 Effects of LF2000 on cell proliferation rate measured by MTT: the cells within 4 passages were seeded onto 96-well plate after digestion by 2.5g/L trypsin and adjusted to a density of 4×10^4/mL,100μL/well. Each group including control had 5 pair wells. After culture for 24 hours, the adherent cells were incubated for 12 hours or 24 hours with LP2000 0 (negative control),5,10,20,40,80mg/L. Then 5g/L MTT was added in each hole. After culture for 4 hours, removed culture medium and added DMSO 100μL for 5minutes at ordinary temperature. Then the absorbance (A-value) at 550nm wavelength of each hole was detected by ELISA Reader. Cell proliferation rate= (A-value of LP2000 group/A-value of negative control group)×100%.

Effects of LF2000 on cell survival rate measured by Trypan blue staining: methods of inoculation and grouping were the same with MTT. 12 hours and 24 hours after LF2000 was added, carefully removed the supernatant and added 4g/L trypan blue a drop in 5 wells of each group, and then observed cells under the inverted phase contrast microscope immediately. Dead cells with broken membrane were stained blue by Trypan blue, while living cells were negative. The number of negative and positive cells in 5 low-power fields of each hole was counted, and mean values were used in statistical analysis. Cell survival rate=(unstained cells number/ total cells number)×100%.

Statistical method Statistical comparisons were made by *t*-test and ANONA with SPSS software.

RESULTS

Culture and Identification of Human Keratocytes Three days after seeding, adherent and monolayer cells migrated from verge of tissue mass, and presented with fusiform shape and round centered nucleus. Seven days after subculturing, cells reached confluence. After cells were seeded on coverslips, vimentin and keratin were immunostained to identify corneal stroma cells.

Effects of LF2000 on Human Keratocytes LF2000 had no obvious effects on proliferation and survival rate of human keratocytes when its concentration was below 20μg/L, which had no statistical significance compared with negative control group ($P>0.05$); while proliferation and survival rate of human keratocytes would decrease significantly when concentration of LP2000 was above 40mg/L for longer than 24 hours ($P<0.05$).

Proliferation and survival rates were higher in 24-hour group than that in 12-hour group when the concentration of LF2000 was 40 or 80mg/L ($P<0.05$).

Proliferation and survival rate of human keratocytes decreased as LF2000's concentration increased (Table 1, 2).

Table 1 Effects of different concentrations of LF2000 on prolifer-ation rate of cultured keratocytes

(proliferation rate $\bar{x}\pm s$ %, n=5)

Concentration of LF2000 (mg/L)	Time	
	12h proliferation rate	24h proliferation rate
0(negative control)	100	100
5	99.0±2.5	98.5±4.3
10	97.2±3.1	96.2±3.3
20	95.2±1.7	93.7±0.9
40	89.3±4.2[a]	80.1±1.9[b]
80	75.4±0.9[a]	69.3±4.9[b]
F value	6.021	7.983
P value	0.007	0.000

[a]$P<0.05$, [b]$P<0.01$ compared with negative control group

Table 2 Effects of different concentrations of LF2000 on survival rate of cultured keratocytes (survival rate $\bar{x}\pm s$%, n=5)

Concentration of LF2000 (mg/L)	Time	
	12h survival rate	24h survival rate
0(negative control)	99.2±3.1	98.9±2.7
5	96.0±1.5	95.5±1.4
10	95.2±0.1	93.2±1.7
20	93.2±1.4	92.7±3.6
40	87.3±2.2[a]	82.0±5.0[a]
80	76.4±3.7[a]	70.2±3.9[b]
F value	5.337	6.382
P value	0.001	0.000

[a]$P<0.05$, [b] $P<0.01$ compared with negative control group

DISCUSSION

Corneal stroma is composed of about 250 intertwining lamellae of collagen fibrils which made up of collagen fibrils parallel arranged into equal diameter. Keratocytes account for the major cell component of stroma, synthesize and secret fibers and also play roles for their alignment and balance. Keratocytes' changes in trauma and repair have important influence on the structure and function of cornea.

Recently, most researches have carried out investigation on corneal repair in trauma. However, some mechanisms about repair still need to be explored. With the rapid development of molecular biotechnology, gene therapy obtained increasing considerations as a completely new therapeutic tool. Gene transfection technology has extensive prospects in diagnosis and therapy of ophthalmology as the basis of gene therapy [1-3]. The key point is the carrier which ensures exogenous gene delivered into target cell. An ideal carrier should deliver objective gene to target cell or tissue under the regulation of proper promoter without inflammatory or immune reaction; it could adjust its expression time in host body according to different therapeutic demands; easy to construct and purify; high titer and without initiation of insertion mutation or wild-type viral pollution [1,2]. Carriers applied in gene therapy include naked DNA (plasmid), cationic liposome, virus, etc [4,5]. Plasmids have low transfection efficiency. Despite high transfection efficiency,

there aexist still some risks in the clinical application because of virus immunogenicity. As a biological method, cationic liposomes are referred to gene therapy *in vivo* owing to the safety, weaker immunogenicity, larger capacity and simplicity to operate, especially the polyvalent cationic liposomes with higher transfection efficiency [6,7]. Based on this experiment, cationic liposome LF2000 did not exert obvious influence on proliferation and survival rate of keratocytes in certain concentration and time. Therefore, LF2000 could be used to deliver target genes into keratocytes because of its intervention in corneal repair in trauma at molecular level, which provides a new efficient therapeutic method for corneal diseases.

REFERENCES

1 Crystal RG. Transfer of genes to humans: early lessons and obstacles to success. *Science* 1995;270(5235):404-410

2 Singh VK, Tripathi P. Gene therapy in ocular diseases. *Indian J Ophthalmol* 2002;50(3):173-181

3 Klebe S, Sykes PJ, Coster DJ, Bloom DC, Williams KA. Gene transfer to ovine corneal endothelium. *Clin Experiment Ophthalmol* 2001;29(5):316-322

4 Murata T, Kimura H, Sakamoto T, Osusky R, Spee C, Stout TJ, Hinton DR, Ryan SJ. Ocular gene therapy: experimental studies and clinical possibilities. *Ophthalmic Res* 1997;29(5):242-251

5 Felgner PL, Gadek TR, Holm M, Roman R, Chan HW, Wen zM, Northrop JP, Ringold GM, Danielsen M. Lipofection: a highly efficient, lipid-mediated DNA-transfection procedure. *Proc Natl Acad Sci USA* 1987;84(21):7413-7417

6 Felgner PL, Ringold GM. Cationic liposome-mediated transfection. *Nature* 1989; 337(6205):387-388

7 Felgner JH, Kumar R, Sridhar CN, Wheeler CJ, Tsai YJ, Border R, Ramsey P, Martin M, Felqner PL. Enhanced gene delivery and mechanism studies with a novel series of cationic lipid formulations. *J Biol Chem* 1994; 269(4):2550-2561