·Basic Research·

Inhibitory effect of propylene glycol mannate sulfate on growth of rabbit lens epithelial cells *in vitro*

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Abstract

• AIM: To investigate the inhibitory effect of rabbit lens epithelial cell (RLEC) survival and growth by propylene glycol mannate sulfate (PGMS) on the rabbit capsular bag *in vitro*.

• METHODS: Capsular bags were prepared from rabbit eyes after extracapsular cataract extraction (ECCE) and incubated in 0.2, 0.4, 0.8g/L PGMS in 2, 5, 10 minutes incubation periods. After treatment, the capsular bags were cultured for 7 days in Dulbecco minimum essential medium (DMEM) supplemented with 50mL/L fetal calf serum (FCS). The specimens were examined with light microscopy and transmission electron microscopy (TEM). Capsular bags without receiving PGMS only served as controls.

• RESULTS: PGMS inhibited the proliferation of RLEC in the manner of concentration and time dependent. At the threshold protocol of incubation in PGMS at 0.8g/L for 5 or 10 minutes, proliferative activity of cells were largely arrested and nearly no RLEC was seen on the posterior capsule ($P<0.05$). Control group had no effect on structure and proliferative activity of RLEC, and the growth proceeded rapidly so that the posterior capsule were totally covered by a confluent monolayer of cell by the end of 7 days. Under TEM, the cells in the control group were tightly arrayed with clearly defined cellular boundary and structure; while cellular deformity and undefined intracellular structure could be seen in the 0.4g/L and 0.8g/L experimental groups.

• CONCLUSION: PGMS can effectively inhibit the proliferation of RLEC.

• KEYWORDS: propylene glycol mannate sulfate; lens epithelial cell; posterior capsular opacification; capsular bag model

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INTRODUCTION

Posterior capsular opacification (PCO) is the most common cause of reduced visual acuity after cataract extraction and intraocular lens (IOL) implantation, with an incidence of up to 50% between 2 months and 5 years after the initial surgery [1]. Several researches had been studied to prevent PCO, propylene glycol mannate sulfate (PGMS), a new low molecular marine drug, is purified from a new drug named propylene glycol alginate sodium sulfate (PSS) by marine drugs and food research institute of Qingdao Marine University of China. The structural organization is similar to heparin in some degree. It had been studied that PGMS could inhibit the proliferation of rabbit lens epithelial cell (RLEC) by many ways, with low activity of anticoagulation and lower tendency of hyperemia compared with heparin[2]. Rabbit capsular bag culture model was proved to be able to mimic the *in vitro* situation after cataract extraction to a large extent. Here, the inhibitory activity and effective concentration of PGMS against the survival and growth of RLEC on the rabbit capsular bag *in vitro*, were investigated.

MATERIALS AND METHODS

Materials PGMS was a gift from Qingdao Zhonglu Haida marine drug company. Dulbecco minimum essential medium (DMEM) was purchased from Gibco company. Fetal bovine serum was purchased from Sijiqing company (Hangzhou). A total of 60 eyes of albino rabbit (animal of either sex under 2 years) were obtained within 1 hour of sacrifice. The animals were divided into 10 groups according to various concentrations of PGMS (0.2, 0.4, 0.8g/L) and various incubation periods (2, 5, 10 minutes). Eyes without receiving PGMS only served as controls.

Preparation of rabbit lens capsules A total of 60 rabbit eyes (animal under 2 years) were obtained within 1 hour of sacrifice. A sham cataract extraction was carried out under a dissecting microscopy. After removal of the corneoscleral disc, the iris was cut from the papillary edge to the ciliary body and each section peeled back beyond the equator of the lens. An anterior continuous curvilinear capsulorhexis (ACCC) approximately 4mm to 5mm in diameter was performed, followed by hydroexpressed of the lens nucleus and cortex with phosphate buffered saline (PBS), any cortical remnant was removed with a pair of forceps. The capsular bags were then dissected by cutting the zonules as

close to the capsule as possible and pinned to sterile non-toxic silicone rings by means of four or five entomological pins through the edge of the capsule to retain their circular shapes. Each capsule was transferred to a petri dish and maintained in DMEM supplemented with 100mL/L fetal calf serum (FCS). The capsules were cultured at 37℃ in a 50mL/L CO_2 atmosphere. The medium was replaced every 3 days.

Drug application prepared Rabbit capsular bags were incubated in various concentrations of PGMS (0.2, 0.4, 0.8g/L) during various incubation periods (2, 5, 10 minutes). After treatment, the capsular bags were washed with DMEM twice, and then cultured for 7 days in DMEM supplemented with 50mL/L FCS. The growth of the RLEC on the posterior capsule was monitored every 24 hours during the culture period by phase-contrast microscopy. Cellular coverage of the posterior capsule and the total area within the rhexis were determined using an eyepiece graticule. The number of squares completely or half covered by cells was recorded on a chart and the percentage coverage was calculated by relating this to the area available within the rhexis. Complete cover or confluence represented as 100%.

Methods

Histological examination After 7 days culture, lens capsules were fixed in 40g/L neutral buffered formaldehyde (NBF) for at least 12 hours. Following fixation, the silicone rings were removed and the capsules were embedded in paraffin wax and 4-5μ m thick section cut. Sections were dewaxed in xylene, dehydrated in a graded series of alcohols, and stained with haematoxylin and eosin. The sections were examined by standard light microscopy and photographed.

Ultramicrostructure examination After 7 days culture, one case of every group was fixed for 12 hours with 30g/L glutaraldehyde, postfixed with 10g/L osmium tetroxide, dehydrated with a graded ethanol or acetone series; and then embedded in epon 618. Ultrathin sections were stained with uranyl acetate and lead citrate. The ultramicrostructure of RLEC on posterior capsule was examined with an electron microscope.

Statistical Analysis SPSS software version 11.5 was used for statistical analysis, group differences of posterior capsule coverage percentage were evaluated by using analysis of variance (ANOVA). $P<0.05$ was considered as statistical significance.

RESULTS

RLEC Growth under Phase-contrast Microscopy In control group, outgrowth was observed across the posterior capsule. After a latent period of 2-3 days, cells continued to move across the capsule in a relatively ordered manner, with large irregular looking cells at the growth edge and a neat

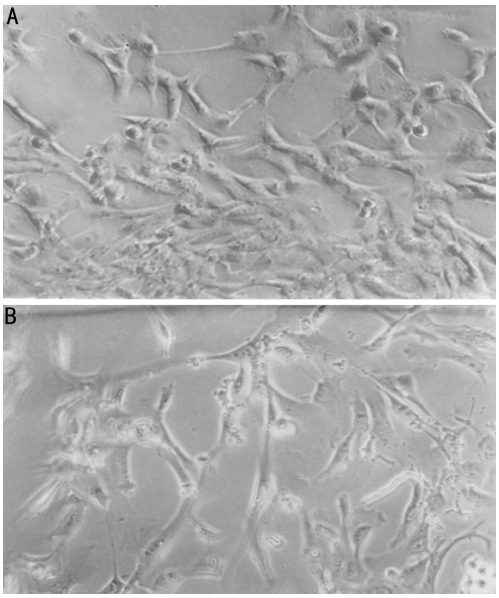

Figure 1 Inhibition of PGMS on RLEC growth on the 7th day (×100) A: Control; B: 0.4g/L PGMS for 5 minutes

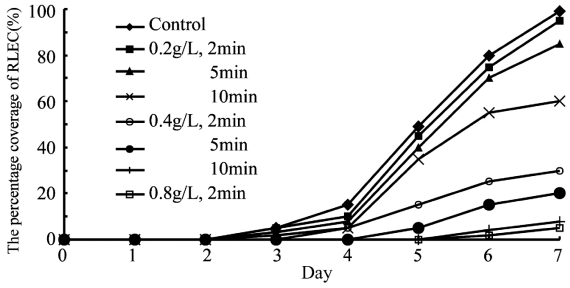

Figure 2 RLEC coverage on posterior capsule in incubation of PGMS

pavement of cells behind (Figure 1A). Growth proceeded rapidly so that the posterior capsule was totally covered by a confluent monolayer of cell at 7th day, thus the percentage coverage was 100%. Wrinkle became visible in the posterior capsule of all samples after cells completely covered the posterior capsule. In some cases, there were many layers of nest appearance cells in equator which might be associated with Soemmering ring in the future. Similar results were seen at low concentration of PGMS (0.2g/L) of each time, in all cases, the posterior capsule had partially or completely opacified. In some cases, the percentage coverage was 100%. In these groups, there was no significant difference in the proliferation rate compared with the control group. An obvious inhibitory effect of PGMS on RLEC was observed at the concentration of 0.4g/L for 2 minutes, partly posterior capsule opacity was visible in only two cases. The percentage coverage was 20% and the difference was significant compared with the control group ($P<0.05$). The inhibition rate increased in a time- and dose-dependent manner. The light-microscopic analysis revealed RLEC with long and narrow appearance had loosely contact with

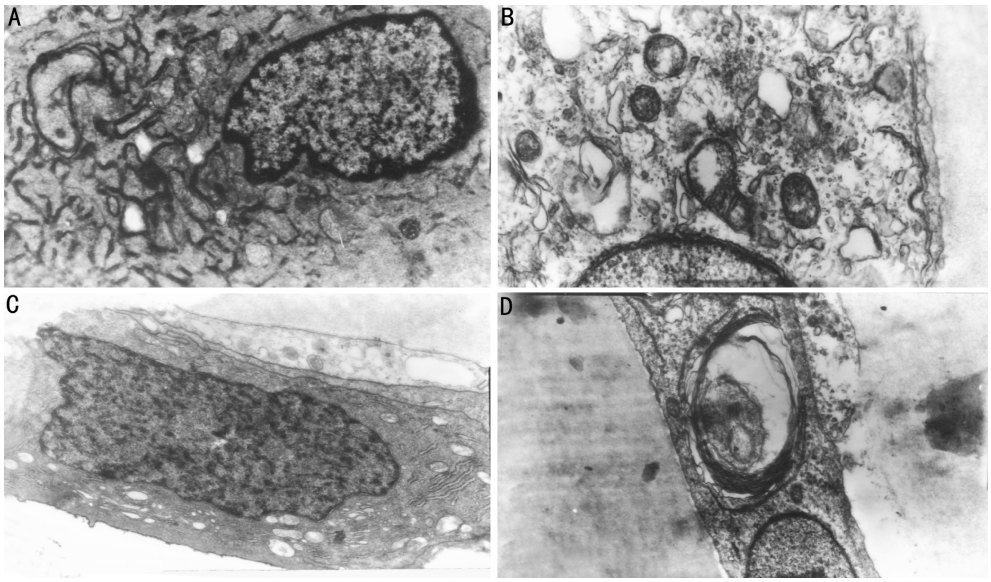

Figure 3 RLEC ultrastructure on capsular bag after 7-day culture (TEM×4 000) A: Control;B: 0.4g/L PGMS for 2 minutes; C: 0.4g/L PGMS for 5 minutes; D: 0.8g/L PGMS for 2 minutes

neighboring cells compared with the control group (Figure 1B). In some high concentration group (0.8g/L PGMS for 5 or 10 minutes) no cell was seen in the posterior capsule (Figure 2).

Histology Alteration After 7 days culture, histology revealed that the posterior capsule was covered by a monolayer cells in both control group and 0.2g/L PGMS of each 3 period groups. RLEC were arranged in a regular pattern, showing an epithelial cell appearance, containing dark staining nuclei and normal cytoplasm. In some cases, coverage cells formed multilayer across the posterior capsule. Rare cells were found on posterior capsule, with loose arrangement and non-tight attachment in 0.4g/L groups of each time and 0.8g/L PGMS for 2 minutes group. In high dose and long time groups, (0.8g/L for 5 and 10 minutes), posterior capsule were smooth and free of cells, in some cases cells on anterior capsule were significantly decreased even disappearing.

Ultrastructure Alteration Under transmission electron microscopy (TEM), the cells in the control group and 0.2g/L PGMS of each 3-period groups had a typical epithelial cell morphology with clearly defined cellular boundary and structure and tight junctions between adjacent cells (Figure 3A); while cellular deformity, cytolysosome of different sizes and cytoplasmic vacuolar degeneration were seen in 0.4g/L and 0.8g/L treated experimental groups. In many cells, nuclear membranes were disappeared, the nuclei appeared condensed; heterochromatin were located on the periphery of cell and medullary sheath degeneration were seen in endochylema (Figure 3B-D).

DISCUSSION

In our previous studies, we designed a mode of LECs culture on capsular bag *in vitro* using silicone ring and observed the proliferation, migration of LECs on the capsule. Compared with simple LECs culture techniques, this model remaining lens capsule bag is simple, novel and effective, without the confounding influence of unknown factors and better mimics the situation *in vivo*. It was concluded that this LECs culture model *in vitro* is useful to support clinical observation and study the biological and pathophysiological aspects of LECs, as well as LECs proliferation, migration, and inhibition. This model will also lead to the development of new experimental measures for PCO prevention. Detailed results of photology, histology, immunohistochemistry and electron microscope about this model can be seen in our published articles [3]. It is general considered that PCO occurs as a result of excessive proliferation and migration and extracellular matrix excretion of residual LECs. Other factors may be contributed to the incidence of PCO, such as blood-aqueous barrier damage, inflammation and immunoreactions after cataract surgery [4]. Pharmaceutical prevention of PCO has always been paying more attention. The ideal drugs should be sufficiently effective in reducing cells proliferation, without toxic effects on cornea endothelial cells, irides, ciliary pigment epithelial cells and other intraocular cells; it also should be suitable for injection into anterior chamber and maintain enough time in an effective dose. Various pharmaceutical means had been developed to remove or destroy the remaining LECs, these attempts include: (1) antimetabolites; (2)antiinflammatory agent,including glucocorticoid and none steroidal hormone; (3)drugs to inhibit fibrinogenesis, such as heparin and fibrinolysin zymoexciter; (4) drugs inhibiting the cell adhesion on lens capsule; (5)

immunotoxins and cytotoxins; (6) gene targeted therapy[5].

Heparin, a well-known drug to inhibit fibrinogenesis, is able to reduce the adhesion of platelet, mechanocyte, monocyte and macrophagocyte, interfere in the activation of granulocyte, decrease intraocular inflammation and fibrin exudation and prevent iris posterior synechia. Secondly, it can inhibit blood coagulation, decrease the formation of fibrin. Thirdly, it also inhibits the proliferation of LECs and fibroblast directly [6]. It must be pointed that although it has many advantages,heparin may induce hyphema postoperatively because of its stronger function of blood coagulation and more easily hemorrhagic tendency, which limit its clinical application. PGMS, a new low molecular heparitin marine drug, is purified from a new drug named PSS by marine drugs and food research institute of Qingdao Marine University of China. The drug is characterized by low molecular mass and simple composition, and is similar to heparin in structural organization. It had been tested that PGMS can shorten the euglobulin lysis time, increase serum fibrinogen degradation product and decrease the level of plasma fibrinogen, which can be concluded that PGMS plays a role in activating the fibrinolytic system. It is also able to decrease thrombus, blood fat and blood viscosity level in serum; inhibit assembly of rhodocyte and platelet; improve microcirculation; eliminate oxyradical and inhibit lipid peroxidation. In additional, anticoagulation effect of this drug is obviously lower than that of heparin, but fibrinolysis capacity is stronger than heparin[7].

Anterior chamber fibrin exudation resulted from blood-aqueous barrier damage is a characteristic inflammation post cataract surgery, which is also involved in the formation of PCO. Heparitin drug, such as heparin, can reduce intraocular fibrin postsurgery by activating profibrinolysin. As we know, fibrin can be made frames for cell proliferation and contraction, promotes lens anterior capsule epithelial cells to migrate towards and invade posterior capsule, which induce PCO finally. Compared with heparin, fibrinolysis capacity of PGMS is stronger. Some results *in vitro* revealed that a certain concentration PGMS can inhibit fibrin exudation dramatically, at the same time, it may stabilize macrophagocyte and decrease its activity. Macrophagocyte is involved in early stage inflammation, it can be converted to fibroblast-like cell and foreign body giant cells in the surface of IOL or posterior capsule, thus produce non-cellular protein membrane, which may absorb more inflammatory cells. Macrophagocyte is involved in inflammation by secreting some biological active medium. Accordingly, PGMS may lessen inflammation of superficial posterior capsule by its influence on macrophagocyte. In our and others previous experiments, we found PGMS can inhibit proliferation of rat LECs *in vitro* culture and rabbit LEC *in vivo* obviously [8]. According experience gained, we discussed the inhibition of RLEC survival and growth on the rabbit capsular bag *in vitro* by PGMS in different concentration and different incubation time. In 0.4g/L for 5 minutes experimental group, PGMS inhibited the proliferation of RLEC dramatically, and with the increase of concentration and time, the effect on proliferation and migration became stronger. At 0.8 g/L for 5 or 10 minutes groups, proliferation activity was largely arrested, nearly no RLEC was seen on the posterior capsule and cellular deformity and undefined intracellular structure could be seen under TEM. Considering no longer operation waiting time, PGMS of 0.8g/L for 5 minutes incubation time may be the most effective to inhibit PCO *in vitro*. The inhibitory effect of PGMS on PCO based on rabbit capsular bag model *in vitro* was discussed, it is necessary to design corresponding experiments to determine if this drug has the same effective on human LEC and is suitable for human intraocular surgery .At the same time, toxic effects of PGMS on cornea, iris, ciliary body, retina and other intraocular tissues are necessary to be studied in further steps.

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