

## EXPERIMENTAL STUDY

# The significance of Akt/NF- $\kappa$ b signaling pathway in the posterior cataract animal model

Shao DW<sup>1</sup>, Zhu XQ<sup>1</sup>, Huo L<sup>1</sup>, Sun W<sup>1</sup>, Pan P<sup>1</sup>, Chen W<sup>2</sup>, Wang H<sup>2</sup>, Liu B<sup>2</sup>

Department of Ophthalmology, Air Force Aviation Medicine Research Institute Affiliation Hospital, Beijing, China. shaodewang2016@sina.com

**ABSTRACT**

**OBJECTIVE:** To establish SD rat posterior capsular opacification (posterior capsular opacification- PCO) animal model, and to detect the expression of Akt/NF- $\kappa$ b signaling pathway in the PCO model.

**METHODS:** 30 healthy SD rats were randomly divided into control group (0d) and the experimental groups (7d and 14 d), there were 10 rats at all time points. All rats (right eye) were treated with the lens capsule, and the inflammatory reaction of the anterior segment of the eye and the occurrence of PCO at different time points were observed under the microscope. The TGF- $\beta$  concentration of humor aquosus was measured at the different time points by ELISA method. Eyeballs were removed after the rats were killed. RT-PCR method was used to detect the gene expression levels of Akt and NF- $\kappa$ b and Western Blot method to detect the protein expression of Akt, p-Akt, NF- $\kappa$ b and p-NF- $\kappa$ b.

**RESULTS:** TGF- $\beta$  concentration, Akt and NF- $\kappa$ b gene expression, and Akt, p-Akt, NF- $\kappa$ b and p-NF- $\kappa$ b protein expression in humor aquosus, increased with the time and the time-dependence was significant.

**CONCLUSION:** Akt/NF- $\kappa$ b signaling pathway may be closely related to the occurrence and development of PCO, which may be related to the role of protein phosphorylation (Fig. 5, Ref. 20). Text in PDF [www.elis.sk](http://www.elis.sk).

**KEY WORDS:** Akt, NF- $\kappa$ b, TGF- $\beta$ , PCO.

**Introduction**

Posterior capsule opacification (PCO), also known as posterior capsule opacification is the most frequent complication of cataract surgery. After cataract surgery, the incidence of 1 ~ 5 PCO D was 50 % ~ 11 %, and the incidence rate was 100 % (1). The study showed that the proliferation, migration and epithelial mesenchymal transition (EMT) of lens epithelial cells (LEC) after cataract surgery were the main reasons for the formation of PCO (2). Transforming growth factor (TGF) can induce the occurrence of EMT. Previous studies have shown that the highest content of TGF- $\beta$  in the water can effectively induce the occurrence of EMT LEC, which is essential for the occurrence of PCO (3). This method has become a cell model for studying PCO (4). Studies have shown that TGF plays an important role in the induction of EMT in retinal pigment epithelial cells (5, 6). But so far, the research on the upstream channel of TGF in PCO is relatively limited. In this study, we used RT-PCR, Elisa and Western Blot methods to study the roles of Akt/NF- $\kappa$ b pathway in PCO pathogenesis.

**Material and methods***Main reagents and instruments*

Department of ophthalmology operation microscope (Suzhou six six visual Polytron Technologies Inc), Surgical instruments in Department of Ophthalmology (Suzhou medical instrument factory), Compound Tropicamide Eye Drops (Shenyang Shengyuan Pharmaceutical Co. Ltd.), Oxybuprocaine Hydrochloride Eye Drops (Santen Pharmaceutical Co., China), Tobramycin and Dexamethasone Eye Ointment (American Alcon Corporation), Atropine Sulfate Eye Ointment (Shanghai General Pharmaceutical Limited by Share Ltd), Hyaluronic acid sodium salt (The victory, Shandong bauschlobm Freda), Anti Sheep anti mouse, immune tissue chemical Kit (Zhongshan Company, Beijing), Anti Akt, anti p-Akt, anti NF- $\kappa$ b antibody (Abochorage Shanghai Trading Co., Ltd.), Rat TGF- $\beta$  ELISA Kit (West Tang Biotechnology Co., Ltd., Shanghai).

*Experimental animals and groups*

30 healthy female adult clean grade SD rats, body weight 240 ~ 260 g, under the microscope observation, exclude the anterior segment lesions. The rats were divided into control group and two experimental groups. All rats were under general anesthesia and lens was removed from the right. In the control group it was performed immediately (0 d), and in the experimental groups 7 days and 14 days after operation.

<sup>1</sup>Department of Ophthalmology, Air Force Aviation Medicine Research Institute Affiliation Hospital, Beijing, China, and <sup>2</sup>Department of Ophthalmology, Air Force General Hospital, Beijing, China

**Address for correspondence:** D.W. Shao, Department of Ophthalmology, Air Force Aviation Medicine Research Institute Affiliation Hospital, Beijing, 100089, China.

## Methods

### Operation method

The operation was performed by the same operator with the improved rat lens capsule. Measuring the body weight 30 min pre-operatively, using 40 g/L hydrate (0.8 ml per 100 g) intraperitoneal injection, general anesthesia 3 times after mydriasis, the rats were fixed on the operating table, Conjunctival sac instillation of 40 g/L Oxybuprocaine Hydrochloride Eye Drops 3 times, each time 1 drop; Saline eye surgery, compound iodine disinfection eye surgery in a clear corneal incision, injecting viscoelastics, keeping anterior chamber; Along the puncture mouth to expand the incision about 180 degrees, Circular capsulorhexis, about 5 mm in diameter, water separation to a nuclear free bag, compression method lens delivery and cortex, incision was sutured to the water dense and the anterior chamber was formed. The conjunctival sac with Tobradex and atropine. Postoperative daily observation in rats with systemic and ocular conditions, PRED Forte eyedrops conjunctival sac eye drops, 3 times a day, 1 drop each time, at night Tu Dian of Tobradex and atropine, a total of 7 days. Respectively on postoperative days 0, 7 and 14 general anesthesia under the microscope observation of anterior segment inflammation and the capsule, fully dilated before each observation and photographic system recorded images.

### Materials and specimen handling

On days 0, 7 and 14 after operation the water in the anterior chamber was extracted under general anesthesia, and kept at  $-80^{\circ}\text{C}$  until the measurements were done. Each group was randomly sacrificed 5 only posterior capsular tissues into the 15 mL EP tube, liquid nitrogen preservation. In each of the remaining five rats eye surgery was performed, lens was fixed in 40 g/L poly formalin, paraffin embedded sliced, patched, hematoxylin eosin stained, neutral gum mounting and observed with optical microscope.

### HE staining

The rats were sacrificed after the removal of posterior capsular tissue that was fixed with par formaldehyde, embedded, sliced; HE stained and observed under light microscope.

### Elisa method

The expression of TGF-beta in water at different time points was collected on days 0, 7 and 14 of water, each hole was added

to the 100 L test sample (activated), and the reaction plate was fully mixed at  $37^{\circ}\text{C}$  for 40 min; Washes the reaction plate and washed thoroughly 4 ~ 6 times and on filter paper dry and India, each hole to join the distilled water and the working fluid in the first antibody, 50 mu L (blank except), fully mixed evenly rear  $37^{\circ}\text{C}$  for 20 min; plate washer *ibid*. Each hole enzyme marked antibody working fluid 100 g/l,  $37^{\circ}\text{C}$  for 10 min; plate washer *ibid*. Each hole adding substrate solution 100 g/l, set  $37^{\circ}\text{C}$  and a dark reaction for 15 min; Each hole was added to 100 L liquid mixture, and the absorbance value was measured at 30 min using the enzyme marker at 450 nm.

### RT-PCR test

The total RNA was extracted from the tissues of each group, and the template for reverse transcription was cDNA. Primer sequence as follow, Akt: F: 5'-GGACAACCGCCATCCAGACT-3'

R: 5'-GCCAGGGACACCTCCATCTC-3'

NF-kb: F: 5'-AATTGCCCCGGCAT-3'

R: 5'-TCCCGTAACCGCGTA-3'

$\beta$ -actin: F: 5'-GCCTCGCTGTCCACCTTCCA-3'

R: 5'-CACCTCACCGTTCAGTTT-3'

Using cDNA as a template, using GAPDH as reference, according to the SYBR Primix Ex Taq II (TaKaRa Kit) was set to 3 holes. Reaction conditions: 95 30s; 95 5s; 60 C 31s, 40 cycles. The  $2^{-\Delta\Delta\text{Ct}}$  value indicates the expression of mRNA.

### Western Blot test

From that of the control group and experimental group lens posterior capsular tissue, adding proper amount of lysate and cracking after 10 000 R/min centrifugation for 5 min, the supernatant was full. Add up 5 folds sample buffer, cooking 5min at  $100^{\circ}\text{C}$ , fully mixed. SDS-PAGE gel configuration, sample, electrophoresis, transfer film. 50 g/L skimmed milk powder room temperature closed 2 h, add a good dilution of a resistance, 4 degrees overnight, TBST wash 2 times, each time 10 min; add a good dilution of two anti, room temperature incubation 1 TBST, H wash 3 times, each time 10 min. Chemiluminescence, developing and fixing. Gel image analysis. GAPDH was used as reference in this study.

### Statistical methods

This study used SPSS 19.0 soft (SPSS Inc., Chicago,USA), all data are expressed as the mean  $\pm$  standard deviation (SD) val-



Fig. 1. HE staining, A: 0 d; B: 7 d; C: 14 d.

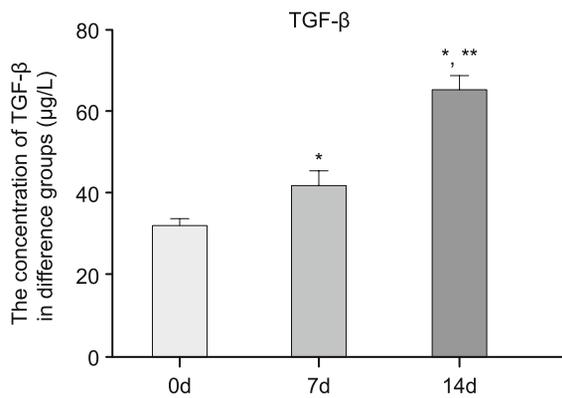


Fig. 2. The concentration of TGF- $\beta$  in different groups (ug/L), \*:  $p < 0.05$ , compared with 0d group, \*\*:  $p < 0.05$ , compared with 7d group.

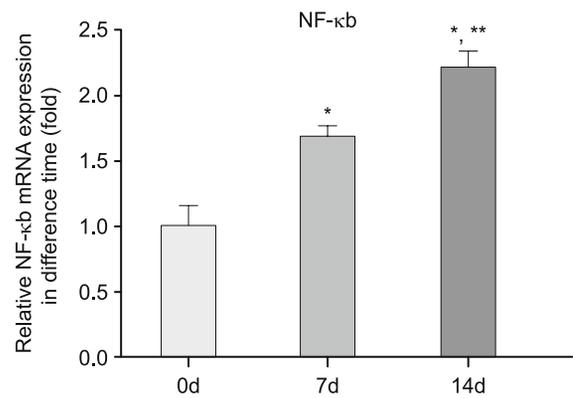


Fig. 4. NF- $\kappa$ B gene expression in different groups (Fold). \*:  $p < 0.05$ , compared with 0d group, \*\*:  $p < 0.05$ , compared with 7d group.

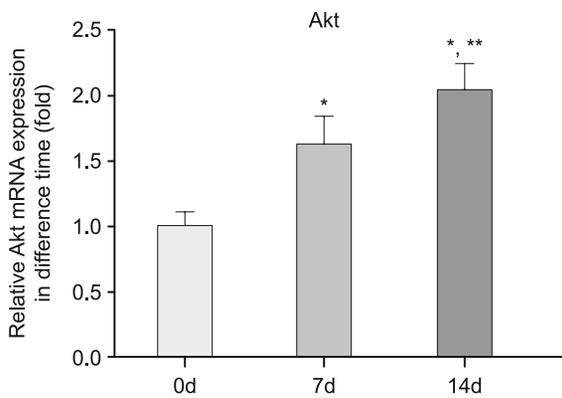


Fig. 3. Akt gene expression in different groups (Fold). \*:  $p < 0.05$ , compared with 0d group, \*\*:  $p < 0.05$ , compared with 7d group.

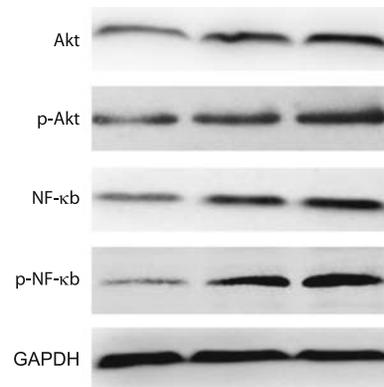


Fig. 5. Relative protein in different groups.

ues ( $\bar{x} \pm s$ ). Using one way ANOVA with LSD analysis methods in differences groups,  $p < 0.05$  considered as statistically significant.

## Results

### HE staining

The lens epithelial cells located in the anterior capsule and equator capsule, simple cuboidal epithelial cells, cell shape, uniform distribution of cells from the crystal capsule wrapped, no lens fiber cell core and at the center of the abnormal (Fig. 1A). The anterior surface of the lens fiber covers the lens epithelial cells, and a large number of non degradable nuclei are seen in the lens fiber cells of the posterior part of the equator to the posterior capsule in 7 d and 14 d with time expanding (Figs 1B and 1C).

### TGF-beta concentration detected in aqueous humor by Elisa method

Postoperative day 0, 7 and 14 after operation in aqueous humor of TGF- $\beta$  expression respectively ( $31.94 \pm 2.05$ ) ug/L, ( $41.92 \pm 3.86$ ) ug/L and ( $65.65 \pm 3.35$ ) ug/L, with the passage of time, real

water TGF- $\beta$  content gradually increased, the differences between the three groups were significant ( $P < 0.05$ , respectively). The data are shown in Figure 2.

### RT-PCR test

The expression levels of different time groups strong posterior Akt and NF kappa B gene, and 0d group compared to day 7 and day 14, Akt and NF- $\kappa$ B gene expression level increased significantly ( $p < 0.05$ , respectively); 7 and 14 days between the two groups, Akt and NF- $\kappa$ B difference was significant ( $p < 0.05$ , respectively). The data are shown in Figures 3 and 4.

### Western Blot test

With the postoperative time, Akt and NF- $\kappa$ B protein expression levels were significantly increased and postoperative days 7 and 14 of Akt, p-Akt, NF- $\kappa$ B and p-NF- $\kappa$ B protein expressions were significantly higher than that of days 0, 7 and 14 between the two groups, Akt and NF- $\kappa$ B differences were significant. The data are shown in Figure 5.

## Discussion

After cataract extraction (including phacoemulsification) or lens injury, residual cortex or LEC hyperplasia, the formation of turbidity, known as PCO. Some previous studies have found that cataract surgery destroyed the blood ocular barrier and various cytokines from the blood into real water, the precise and orderly LEC normal differentiation program is disrupted, large amounts of cytokines stimulation residues in the anterior capsule and equatorial portion of LEC. The proliferation, migration, and EMT and collagen fibers in the extracellular matrix of the deposition of large amounts, resulting in posterior capsular wrinkle, shrink and turbidity. At present, many studies have confirmed that the abnormal deposition of LEC and extracellular matrix induced by TGF- $\beta$  is the key to the formation of PCO (7, 8).

A variety of signaling pathways are involved in the occurrence and development of PCO (9–11). Akt/NF- $\kappa$ B signaling pathway is a common signal transduction pathway in eukaryotic cells. It is involved in cell polarity, cell division, proliferation and apoptosis, cell adhesion and migration, and so on (12, 13). Related studies confirmed that the inhibition of TGF- $\beta$  upstream protein can effectively inhibit the collagen gel contraction induced by TGF- $\beta$  and the expression of type I collagen (14, 15).

This study found that through the establishment of animal model of SD rat PCO, aqueous TGF  $\beta$  content increased, posterior capsular Akt, p-Akt, NF  $\kappa$ B and p-NF- $\kappa$ B, expression increased, Akt/NF- $\kappa$ B signaling pathway is activated so as to promote the proliferation of LEC and the lens transparency effect. Previous studies have confirmed that TGF- $\beta$  can regulate the related genes and proteins by activating the RhoA signaling pathway, which can induce the production of tension fibers and EMT (16–18). Studies had confirmed that Akt/NF-nuclear factor  $\kappa$ B can induce the formation of TGF- $\beta$  (19, 20), However, it is not clearly that Akt/NF- $\kappa$ B signaling pathway has a role in PCO.

The results of this study showed that TGF- $\beta$  was activated in the aqueous humor after cataract surgery in rats. The concentration of 0d was gradually increased with the passage of time, and the concentration of 0dTGF- was the lowest, and 14d reached the peak after operation. We found that the expression of 0d was the lowest, and then gradually increased, and 14d reached the peak after operation depend on respectively measuring gene expression of Akt and NF- $\kappa$ B and protein expression of Akt, p-Akt, NF- $\kappa$ B and p-NF- $\kappa$ B by RT-PCR and Western blot methods. According to the experimental results, we can see that the postoperative rats of posterior lens capsule on Akt and NF  $\kappa$ B expression and at the same time, the main rooms water TGF  $\beta$  expression was consistent, showing a time dependent trend, which can be inferred in rats after cataract surgery in aqueous humor of TGF  $\beta$  is activated, following the activation of  $\kappa$ B Akt/NF- B signal pathway and induced EMT of lens, which promote the development of PCO.

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