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Original Article

Echistatin prevents posterior capsule opacification in diabetic rabbit model via integrin linked kinase signaling pathway

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Abstract: Purpose: To investigate the effect of disintegrin echistatin on integrin linked kinase (ILK) and subsequent PI3-K/Akt and ERK1/2 signaling pathways in the posterior capsule opacification (PCO) model of diabetic rabbit. Methods: 56 rabbits were injected alloxan to model diabetic. Then they accepted lens extraction surgery and randomly and intraoperatively injected distilled water (control group; n = 28) or 10.0 mg·L^{-1} echistatin (echistatin-treated group; n = 28) into the anterior chamber. Each group was subdivided into ten days group (n = 14) and six weeks group (n = 14) respectively. The PCO severity was evaluated with a slit lamp microscope and light microscope for 10 days and 6 weeks postoperatively. The levels of ILK in the posterior capsule were determined by Q-PCR, Western blotting and Immunohistochemistry. Akt and ERK1/2 phosphorylation were analyzed by Western blotting. Results: 10 days and 6 weeks after surgery, the grades of PCO in the echistatin-treated group were lower than the control group. The lens epithelial cells (LECs) in the posterior capsule of echistatin-treated eyes had decreased degrees of proliferation and migration than the control group. And no significant side effects appeared after treated with echistatin. Echistatin could significantly reduce the expression of ILK in terms of both mRNA and protein levels. The phosphorylation levels of Akt and ERK1/2 were decreased in the echistatin-treated group compared with the control group. Conclusions: Echistatin could inhibit postoperative PCO occurrence and development in diabetic rabbit eyes, which may be related to down-regulation the expression of ILK and inhibition the PI3-K/Akt and ERK1/2 pathways.

Keywords: Echistatin, integrin linked kinase, PI3K/Akt signaling, ERK1/2 signaling, posterior capsule opacification, diabetic rabbit

Introduction

Posterior capsular opacification (PCO) is the most common complication of cataract surgery [1-3]. PCO is also a frequent and important complication noticed in diabetics after cataract surgery [4-7]. It has been reported that diabetic patients develop significantly more severe PCO after cataract surgery than nondiabetic patients [4, 8, 9]. In diabetic patients, a clear posterior capsule, it is not only for good visual acuity but also for fundus visualization, which may be needed for vitreous surgery or even retinal photocoagulation treatment, such as diabetic retinopathy (DR) and macular edema. Thus, an effective method to prevent the PCO, especially for diabetic patients, is urgently needed.

Echistatin, which belongs to the disintegrin family, isolated from the venom of *Echis carinatus,* is composed of two isoforms with molecular weights of 5.2-5.4 kDa [10]. It has been found that echistatin is a potent inhibitor of platelet aggregation [11, 12]. It also has been found that echistatin significantly decreased Insulin-like growth factor 1 (IGF-1) stimulated phosphorylation of PI3-K and subsequent signaling [13, 14]. And our previous studies demonstrate that 10.0 mg·L^{-1} echistatin can inhibit lens epithelial cells (LECs) proliferation, migration and epithelial-mesenchymal transition (EMT) in vivo with high glucose [15, 16]. We also found that PCO is gradually aggravated with time prolonged, the LECs proliferate at 10 days and reach a peak at 6 weeks after lens extraction in diabetic rabbit eyes [17]. However, as yet, it is not clear that echistatin inhibit postoperative LECs action through which signaling pathways.

Integrin linked kinase (ILK) is a serine-threonine kinase and localized at the cell-matrix interface [18]. It acts as an intermediate to link extracellular integrin signals to intracellular signaling pathways. It has been reported that, subsequent to cataract surgery, ILK could play a role in the requisite EMT of LECs, which contributes to the development of PCO [19]. Moreover, activated ILK can regulate multiple integrin-mediated signal transduction pathways such as the PI3K/Akt and ERK1/2 pathways [20]. And PI3K/Akt and ERK1/2 pathways are thought to primarily play a role in lens cell proliferation and differentiation [21, 22], which promote the PCO occurrence and development. In view of the role of ILK and its downstream PI3K/Akt and ERK1/2 pathways in PCO formation, the present study was therefore designed to investigate the effect of disintegrin echistatin on ILK and its downstream PI3-K/Akt and ERK1/2 signalling pathways in LECs after extracapsular lens extraction in diabetic rabbit model.

Materials and methods

Rabbits

Fifty-six healthy New Zealand rabbits, mixed gender, 12 weeks of age, weighing 2.4-2.8 kg, were purchased from the Experimental Animal Center of Guangxi Medical University (Guangxi, China). The eyes were normal in slit lamp examination. All animal studies were performed according to the Association for Research in Vision and Ophthalmology Statement on the use of animals in Ophthalmic and Vision Research, and were approved by the Animal Ethics Committee of Guangxi Medical University.

Induction of diabetes

After 8 hours of fasting for solids and liquids, 90 $mg \cdot kg^{-1}$ Alloxan monohydrate (A7413, Sigma, USA) were injected via the ear margin vein of New Zealand rabbits (n = 56) to model diabetic rabbits [23]. The criterion of successful modeling is the rabbits' blood sugar higher than 12 $mmol \cdot L^{-1}$ for 2 weeks. For the blood glucose above 16 $mmol \cdot L^{-1}$, we gave different units of insulin glargine injection (Sanofi-Aventis, France) in subcutaneous injections, according to different blood glucose levels. Making sure the blood sugar was controlled between 12 to 16 $mmol \cdot L^{-1}$.

Diabetic rabbits grouping and treatment

According to our previous studies [16, 17], fifty-six eyes (right eyes) from 56 diabetic rabbits, in accordance with the random number table, were divided into the control group (n = 28) and the echistatin-treated group (n = 28). Then each group was subdivided into ten days group (n = 14) and six weeks group (n = 14) respectively. After 8 hours of fasting and water-deprivation, rabbits were anaesthetized with sodium pentobarbital 3% (1.0 $ml \bullet kg^{-1}$). Then extracapsular lens extraction (ECLE) were performed in all right eyes for PCO models of diabetic rabbits. At the end of the operation, the echistatin-treated group was injected 0.2 mL 10.0 $mg \cdot L^{-1}$ echistatin (E1518, Sigma, USA) into the anterior chamber, and the control group received 0.2 mL distilled water. One surgeon performed all surgeries. Postoperatively, guttaeatropinisulfatis 1%, tobramycin 0.3%, dexamethasone 0.1% eye drops was instilled four times daily, and tetracycline cortisone eye ointment was used at night.

Observation and assessment criteria

Diabetic rabbit cornea, anterior chamber and the grades of PCO were examined 1 day, 3 days, 7 days, 10 days and 6 weeks postoperatively in the two groups by slit-lamp microscope. The anterior chamber inflammation and corneal edema were graded on a scale of 0-3: 0 = absent; 1 = mild; 2 = moderate; 3 = severe reaction [24]. The PCO were graded from 0 to 3+: 0 = no opacification; 1+ = minimal opacification, fundus visualized; 2+ = moderate opacification, fundus partially obscured; 3+ = severe opacification, fundus completely obscured [25]. The grade was rechecked in case of disagreement between the two observers.

Tissue collection

Rabbits were sacrificed at 10 days or 6 weeks after operation. The right globes (n = 2 for each group) were enucleated then immediately fixed in 10% neutral buffered formalin for histological and immunohistochemistry processing. The posterior lens capsules with adherent LECs (n = 12 for each group) were obtained by continuous curvilinear capsulorhexis from other right globes. Then they were snap frozen and stored at -80°C for further RNA or protein extraction.

Histopathology and immunohistochemistry

The enucleated eyes (n = 2 for each group) were embedded in paraffin, and cut into 4-μm-thick sections. Then sections were dewaxed and rehydrated to water, stained with haematoxylin and eosin (H&E) to observe pathologic changes. As well as immunohistochemical was used to localize ILK in the posterior lens capsule. Antigen retrieval was carried out by heat-mediated sodium citrate antigen for 10 min. Then sections were blocked in normal goat serum (Solarbio, China) for 30 min before treated overnight at 4°C with the rabbit anti-integrin linked ILK (ab74336, Abcam, USA, diluted at 1:500). Antibodies bound sections were visualized with 3,3-diaminobenzidine (DAB) (Maixin Biotech, China) and hematoxylin counterstain. The images of stained sections were collected with a microscope (BX53, OLYMPUS, Japan).

Quantitative PCR

The total RNA was isolated from the posterior lens capsules (n = 6 for each group) using TRIzol Reagent (Invitrogen, USA) and the concentration of total RNA was quantified by NanoDrop2000 (Thermo Fisher, USA). Then the RNA was converted into cDNA using a PrimeScript RT reagent Kit with gDNA Eraser (RR047A, Takara, Japan), according to the manufacturer's protocol. Real-time PCR was performed using SYBR®Premix Ex TaqII (RR820A, Takara, Japan) on a LightCycler® 480 real-time PCR system (Roche, USA). The thermocycling conditions were as follows: 1 cycle for 30 sec at 95°C for initial denaturation; 40 cycles of 5 s at 95°C and 30 s at 60°C for amplification; melting curves analysis was carried out to verify the absence of primer dimers and/or non-specific PCR products. The primer sense/antisense sequences are ILK, 5'-ACATCGTGGTGAAGGTGC-TG-3'/5'-GTAATGAG GGTTGGGTGAGGAG-3'; GA-PDH, 5'-CCACTTTGTGAAGCTCATTTCCT-3'/5'-TC-GTCCTCCTCTGGTGCTCT-3'. The relative mRNA of ILK was normalized to endogenous control GAPDH and then expressed as fold induction over baseline.

Western blotting

Protein was extracted from the posterior lens capsules (n = 6 for each group) in lysis buffer [26] at 4°C for 30 min. Protein concentration was determined using a BCA Protein Assay Kit (Beyotime Biotechnology, China). Then, protein samples were boiled and protein (30 μg/well) was subjected to 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis. Following electrophoresis, the gel-separated proteins were transferred onto polyvinylidene difluoride (PVDF) membranes that were subsequently incubated for 1 hour at room temperature with 5% (w/v) skim milk powder in TBS-T (0.1% Tween 20 in Tris-buffered saline, TBS). The blocked membrane was incubated over night at 4°C with ILK (diluted 1:2000) as described above and Akt (PAB15422, Abnova, Taiwan, diluted at 1:3000), phospho-Akt (phospho T^{34}) (ab23509, Abcam, USA, diluted at 1:1000) , p44/42 MAPK (Erk1/2) (#4695, Cell Signaling Technology, USA, diluted at 1:1000) and phospho-p44/42 MAPK (Erk1/2) (Thr^{202}/Tyr^{204}) (#4370, Cell Signaling Technology, USA, diluted at 1:2000), mouse anti-β-actin (sc-47778, Santa Cruz, USA, diluted at 1:500). All primary antibody incubations were followed by the secondary antibodies [IRDye 800CW Goat (polyclonal) anti-mouse IgG (H+L) #926-32210; and IRDye 800CW Goat (polyclonal) anti-rabbit IgG (H+L) #926-32211; Li-Cor Biosciences, USA)] diluted 1:10000 for 2 hours at room temperature. The complex was visualized with a Li-Cor Odyssey Infra-Red Imaging System (Li-Cor Biosciences, USA) according to the manufacturer's specifications.

Statistical analysis

All statistical analysis was performed with SPSS 16.0 software (SPSS, USA). Numerical variable was expressed as mean ± standard deviations (SD), and was analyzed using a Student *t* test after the demonstration of homogeneity of variance with an F test. Nonparametric test was conducted with ranked data. Two-tailed *P* values <0.05 were considered statistically significant.

Results

No significant difference of postoperative inflammation in the both groups

After surgery, varying intensity of anterior chamber inflammation appeared in both groups of operation eyes for the first three days, and almost entirely restored to normal 7-10 days postoperatively. In addition, different degree of

Table 1. Comparison of postoperative reaction in the both groups (n = 14)

Group	Postoperative reaction	1 day	3 days	7 days	10 days	6 weeks
Control	Anterior chamber inflammation	2.07±0.62	1.79±0.70	0.93±0.62	0.14±0.36	0±0
	Corneal edema	1.79±0.43	1.21±0.58	0.29±0.47	0.07±0.27	0±0
Echistatin-treated	Anterior chamber inflammation	2.14±0.54	1.86±0.66	0.86±0.66	0.21±0.43	0±0
	Corneal edema	1.86±0.36	1.29±0.61	0.21±0.43	0±0	0±0

No significant different in comparisons of both groups (*P*>0.05); Nonparametric test.

Table 2. Comparison of PCO grades in the both groups (n = 14)

Odrich grade	10 days		6 weeks	
	Control	Echistatin-treated	Control	Echistatin-treated
0	6	11	0	2
1+	8	3	3	10
2+	0	0	7	2
3+	0	0	4	0

corneal edema was found in some eyes of both groups, and cornea gradually restored transparent at 5-7 days postoperatively. There was no significant difference in anterior chamber reaction and corneal edema in both groups postoperatively (*P*>0.05, **Table 1**).

Echistatin reduces the grades of PCO

As shown in **Table 2**, at 10 days after-operation, 8/14 control eyes had minimal opacification (grade 1+), accounting for 57%, and 6/14 eyes had no PCO, accounting for 43%. While there were only 3/14 echistatin-treated eyes at grade 1+, accounting for 21%, and 11/14 eyes had no PCO, accounting for 79%. Although there was no significant difference in PCO grades in the two groups (*P* = 0.057). At 6 weeks postoperatively, all control eyes had PCO, accounting for 100%. Among them, 11/14 eyes had reached at grade 2+ or 3+ (7/14 or 4/14), showing significant opacity that most or completely obscured fundus (**Figure 1A**), and 3/14 eyes at grade 1+. By contrast, 2/14 echistatin-treated eyes had no PCO, accounting for 14%, and 12/14 eyes had PCO, accounting for 86%. Among them, 10/14 eyes at grade 1+, showing localized capsular folds and fibrosis (**Figure 1B**), and 2/14 in grade 2+. A statistically significant difference was noted between the two groups (*P* = 0.001), the PCO grades for the echistatin-treated group were significantly lower than those for the control group.

Echistatin suppresses the migration and proliferation of LECs and has no significant side effects on other ocular tissues

H&E staining indicated that the migration and proliferation of LECs in the control group were higher than the echistatin-treated group at 10 days and 6 weeks postoperatively, as showed in **Figure 2A**. In addition, especially at 6 weeks after surgery, multilayered LECs on the posterior capsule were noted in the control group, and their contraction produced numerous tiny wrinkles in the posterior capsule. While there was a small number of LECs noted in the bow region of the echistatin-treated group. H&E staining also indicated that the structure of cornea and retina were well preserved at 10 days and 6 weeks postoperatively after treated with echistatin. Corneal endothelial cell and retina cell were smooth, continuous, intact, and there were no evident findings of inflammation. Other complications were not observed too (**Figure 2B**).

Echistatin inhibits the expression of ILK

To investigate the effect of echistatin on ILK expression, the posterior lens capsules were removed from the eyes at 10 days or 6 weeks after surgery. Then we assayed for protein and mRNA of ILK using western blotting and quantitative PCR. We found that echistatin could inhibit the expression of ILK. The control group was higher 3.63-fold than the echistatin-treated group on the protein level at 10 days (35.948±2.442 *vs.* 9.898±3.375; n = 6; *P* = 0.000) and 1.86-fold at 6 weeks postoperatively (9.035±2.899 *vs.* 4.847±2.018; n = 6; *P* = 0.016) (**Figure 3B**). Quantitative PCR confirmed that level of ILK mRNA was significantly higher in the control group than in the echistatin-treated group at 10 days (0.867±0.223 *vs.* 0.487±0.138; n = 6; *P* = 0.005) and 6 weeks

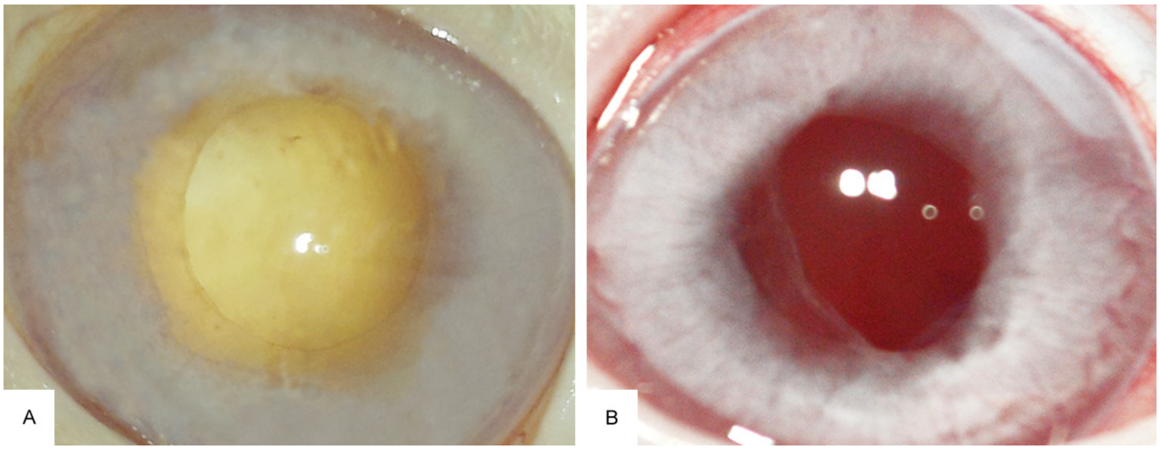


Figure 1. External photography of diabetic rabbit eyes at 6 weeks after surgery. A. Control eye, grade 3+; B. Echistatin-treated eye, grade 1+.

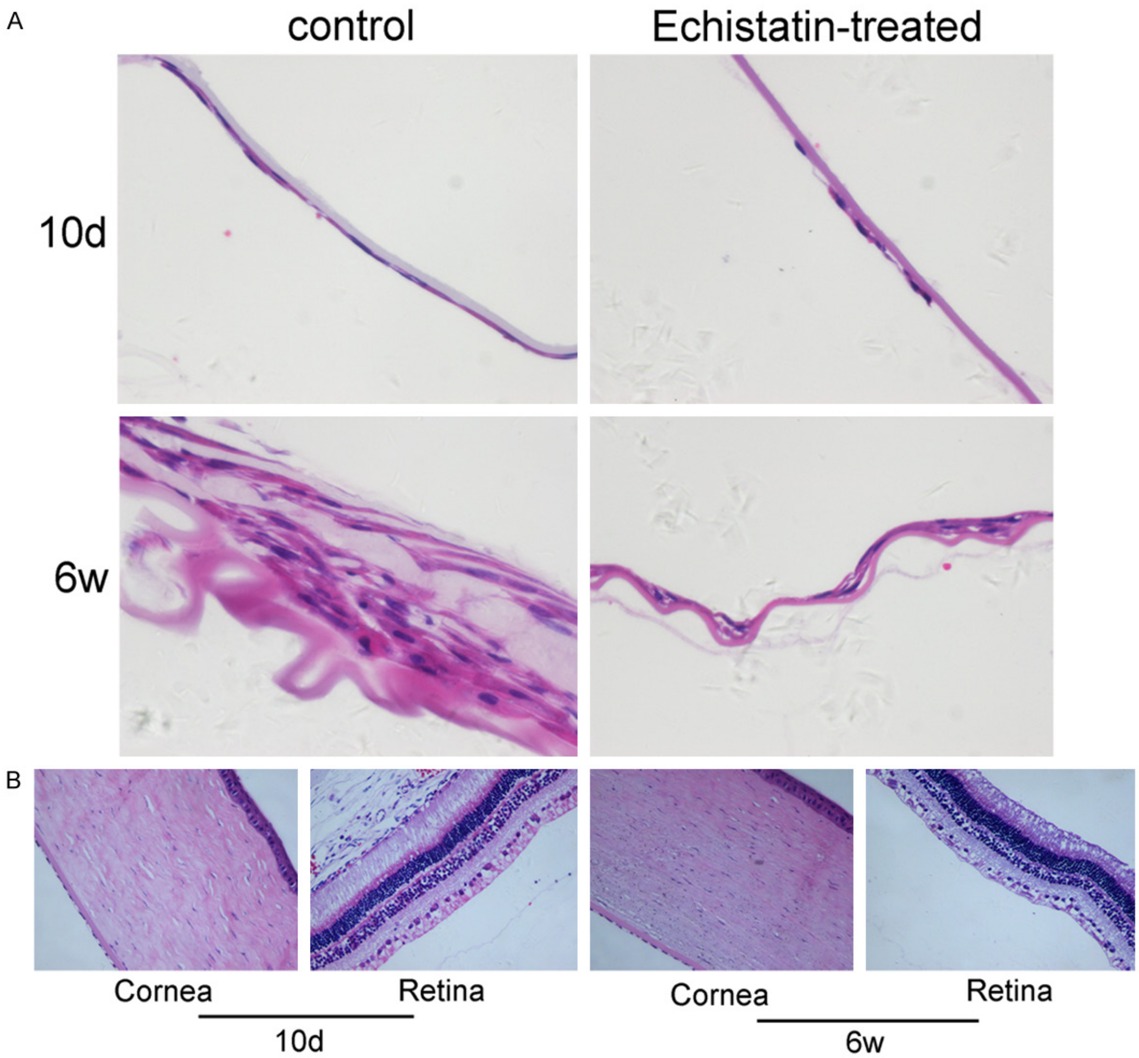
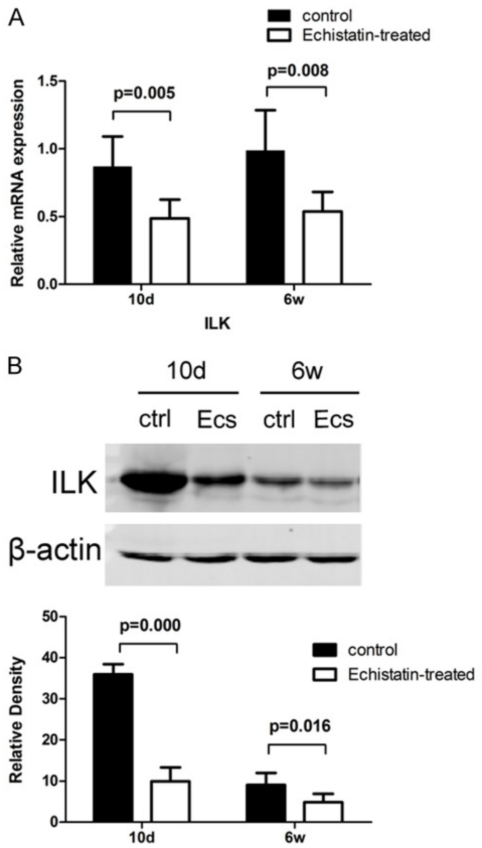


Figure 2. Histopathologic analysis of diabetic rabbit eyes at 10 days and 6 weeks after surgery. A. The proliferation and migration of LECs in the echistatin-treated eyes were markedly inhibited than in the control eyes, no matter 10 days or 6 weeks postoperatively. (Hematoxylin and Eosin staining, ×400). B. No significant changes were observed

in the cornea and retina, that the structure was well preserved after treated with echistatin. (Hematoxylin and Eosin staining, ×200). 10 d = 10 days postoperatively; 6 w = 6 weeks postoperatively.



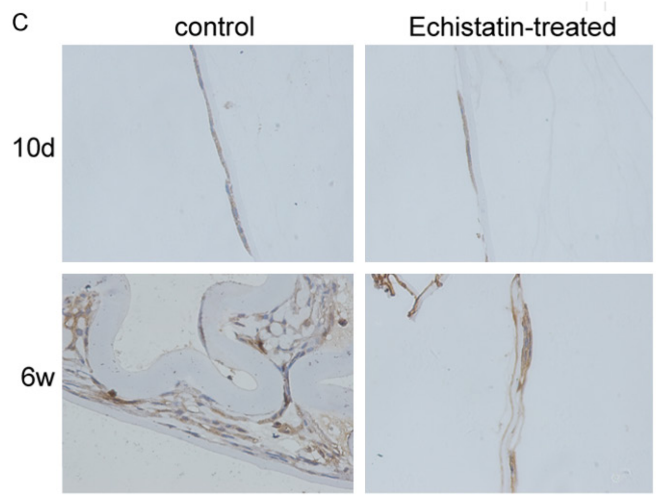


Figure 3. Expression of ILK in diabetic rabbit eyes was inhibited by echistatin at 10 days and 6 weeks after surgery. A. Quantitative PCR for ILK, with GAPDH as a normalized control. B. Western blotting for ILK, with β-actin as a loading control. C. Immunohistochemistry analyses (×400). Each experiment was repeated at least three times with similar results. Quantitative PCR and western blotting results are shown as fold-changes compared with the control. The values are mean ± S.D. (n = 6). Student *t* test. Ctrl = control group; Ecs = Echistatin-treated group; 10 d = 10 days postoperatively; 6 w = 6 weeks postoperatively.

postoperatively (0.985±0.301 *vs.* 0.537±0.145; n = 6; *P* = 0.008) (**Figure 3A**). To define further the expression pattern of ILK in the posterior lens capsule, we embedded whole globes for sectioning and immunohistochemisty. As showed in **Figure 3C**, ILK in LECs was localized in the cytoplasm and associated with cell membranes. Control eyes showed marked cytoplasmic staining of ILK, while the immunoreactivity of LECs in echistatin-treated eyes was significantly reduced for 10 days and 6 weeks after surgery.

Echistatin inhibits the activation of Akt and ERK1/2

To quantify changes in Akt and ERK1/2, western blotting was used to examine the phosphorylation status of Akt and ERK1/2 after treatment with echistatin. The phosphorylation levels of Akt was significantly decreased in the echistatin-treated group at 10 days (1.115±0.291 *vs.* 1.650±0.195 in the control group; n = 6; *P* = 0.004) and 6 weeks after-operation (1.108±0.361 *vs.* 1.802±0.411 in the control group; n = 6; *P* = 0.011) (**Figure 4B**), with an unchanged total Akt at 6 weeks postoperatively (*P* = 0.508), but a decreased level of total Akt at 10 days postoperatively (*P* = 0.008). The phosphorylation levels of ERK1/2 was effectively suppressed in the echistatin-treated group at 10 days (0.195±0.093 *vs.* 0.363±0.112 in the control group; n = 6; *P* = 0.018) and 6 weeks postoperatively (0.087±0.022 *vs.* 0.138±0.043 in the control group; n = 6; *P* = 0.026) (**Figure 4C**), whereas there was no change in total ERK1/2 expression (*P* = 0.208 and 0.731, respectively). These data implied that the Akt and ERK1/2 pathways could be blocked by echistatin after surgery.

Discussion

In the present study, our results indicate that incidence of PCO was significantly reduced after treatment with echistatin in diabetic rab-

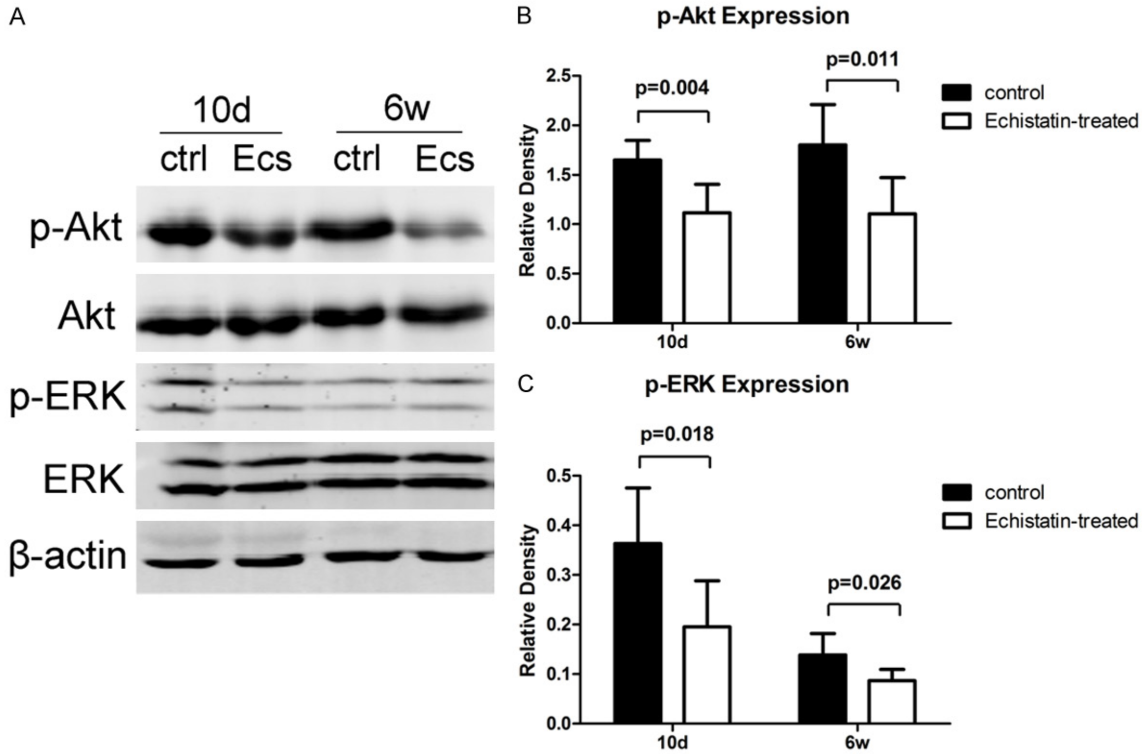


Figure 4. Echistatin inhibited the activation of Akt and ERK1/2 in diabetic rabbit eyes at 10 days and 6 weeks postoperatively. A. The lysates were subjected to western blotting analysis with Akt, phosphor-Akt, ERK1/2 and phosphor-ERK1/2. β-actin was used as the loading control. B. Phospho-Akt/Akt ratio. C. Phospho-ERK/ERK ratio. The experiment was repeated at least three times with similar results. The values are mean ± S.D. (n = 6). Student *t* test. Ctrl = control group; Ecs = Echistatin-treated group; 10 d = 10 days postoperatively; 6 w = 6 weeks postoperatively.

bit eyes. And no significant side effects appeared in the echistatin-treated eyes. Injecting echistatin into the anterior chamber could significantly down-regulate the expression of ILK in terms of both mRNA and protein levels in the early stage of PCO formed (10 days postoperatively) and the most obvious period (6 weeks postoperatively) in diabetic rabbit eyes. The phosphorylation levels of Akt and ERK1/2 were decreased, that the Akt and ERK1/2 signaling pathways could be also inhibited by using echistatin at the two time points respectively.

Previous evidence has been demonstrated that integrins are the major receptor family that mediates binding of cells to the extracellular matrix (ECM). While integrin receptors also function as the major transducers of signals between the cell and the ECM. The signaling induction by integrins is crucial to cell adhesion, proliferation, morphogenesis, differentiation and survival [20, 27]. However, as cell signaling receptors, integrins lacking endogenous enzyme activity, function by contacting with the downstream protein kinase, then trigger subsequent signaling events in cytoplasmic [28]. It has been found that many integrin members are present in LECs [29, 30], and some integrins have been reported to upregulate in LECs after surgery [31]. The targeted deletion of β1-integrins in the lens leads to loss of the LECs phenotype [32]. And double null mutations of α3 and α6 integrin result in a significant lens dysmorphogenesis in the mouse [33]. So integrins play an important role in both lens development and homeostasis.

ILK is a key regulator of integrin-mediated signal transduction pathways. It functions critically as an adaptor protein. ILK binds the cytoplasmic tail of β1 and β3-integrins, and couples them to the actin cytoskeleton [34]. One finding suggests that it cooperate with β1-integrins to control lens cell survival and link lens fibers to the surrounding extracellular matrix [27]. And the loss of ILK in the developing lens results in aberrant matrix assembly [35]. Meanwhile, recent studies imply that ILK has an increasing expression in response to high glucose [36, 37]. ILK can further activate downstream integrin signal transduction pathways, such as the PI3-K/Akt and ERK1/2 pathways. Activation of PI3-K/Akt and ERK1/2 pathways is required for proliferation, migration and differentiation of residual LECs after lens extraction that plays an important role in the pathologic process of PCO [20, 38-40]. In addition, past studies indicated that the inhibition of Akt and ERK1/2 pathways may prevent the formation of PCO [41]. Therefore, interfered with integrin function then decreased the increasing levels of ILK, reduced phosphorylation levels of Akt and ERK1/2 may influence the pathogenesis of PCO.

We treated PCO of diabetic rabbit with echistatin, a member of disintegrins, which can specific bind to integrins and block the interaction integrins with their matrix ligands. The 10.0 $mg{\cdot}L^{-1}$ echistatin were injected into the anterior chamber after the lens extraction. We found that grades of PCO in the echistatin-treated eyes were lower than the control eyes after surgery. Especially at 6 weeks postoperatively, echistatin-treated and control eyes had significantly different grades of PCO ($P<0.01$). Histopathological examination confirmed that the echistatin-treated eyes had significantly less proliferation and migration of LECs than the control eyes at the 10 days and 6 weeks postoperatively. These findings are consistent with those from a previously study reported that disintegrin salmosin significantly inhibits the migration, proliferation and attachment of bovine LECs and rabbit lens cells *in vitro* and *in vivo* [42]. We also found that the expression of ILK, using both mRNA and protein probes, were markedly decreased in the echistatin-treated group at the 10 days and 6 weeks after surgery. In addition, the phosphorylation levels of Akt and ERK1/2 were effectively suppressed, the fractions of p-Akt/Akt and p-ERK1/2/ERK1/2 were decreased. In fact, it was found that SPARC (secreted protein acidic and rich in cysteine) interaction with integrin β1 and enhances ILK activity during the induction of stress in cultured cells, which was required for the survival of LECs. Whereas inhibition of integrin β1 and ILK resulted in increased apoptosis of LECs [43]. It was also noted that the α7β1 integrin-ILK complex can stimulate phosphorylation of Akt, resulting in increased muscle growth in dystrophic mice. And the α7β1 integrin-ILK complex can also decrease the pro-apoptotic actions of BAD and enhancing cell survival, via ERK1/2 pathway [44]. However, deleted ILK from the developing lens can alter Akt and ERK reactivity and particularly depress the Akt activity, resulting in increasing apoptosis and abnormal fiber differentiation in lens [20]. Our study found that echistatin reduces the grades of PCO in diabetic rabbits, providing a suppression effect for PCO of diabetic rabbits. This may be due to inhibition of ILK increasing expression then block the PI3-K/Akt and ERK1/2 signaling pathways in diabetic rabbit eyes.

An interesting finding to come from this *in vivo* model with high glucose was that the ILK and phosphor-ERK1/2 proteins concentration were determined a greater 2 to 3.98-fold increase at 10 days postoperatively than 6 weeks postoperatively in the both groups (data not shown). In fact, it was found in previous studies that the aqueous humor protein concentration was higher in operated rabbit eyes compared to normal eyes [45]. The most likely explanation is that the rabbit undergone an inflammatory response following surgery, which lead the protein concentration to be greater for 10 days postoperatively than 6 weeks after surgery. However, the raised concentration in their study was different from our data. It may be the differences of comparable objects, that two operated eyes in our data while one operated eye and one normal eye in their study. And we detected the protein concentration of the posterior capsules, whereas it was aqueous humor protein concentration in their study. Moreover, it was showed that posterior synechia was seen more often in diabetic patients [46] and the blood-aqueous barrier breakdown to be more severe in the eyes with diabetes [4]. So the diabetic rabbit eyes in our study might have a more severe inflammatory response after surgery. And our study also showed that it was just the phosphor-ERK1/2 protein increase, no phosphor-Akt, for 10 days postoperatively than 6 weeks postoperatively. The mechanism is unknown. It may be that the ERK1/2 and Akt signaling pathways have different reaction to inflammatory. There might also the reason that not only the phosphorylation levels of Akt was significantly decreased in echistatin-treated group, but also the total Akt level was decreased for 10 days postoperatively. Contrastively, previous study showed that the reactivity for total Akt appeared unchanged, though the reactivity for phosphor-Akt was greatly reduced [20]. Since data on our study cannot explain these very clearly. At the current time, there is still much to learn about the pathogenesis of inflammatory between Akt and ERK1/2 pathways is different in vivo with high glucose.

To our knowledge, this study is the first report evaluating the effect of echistatin on the level of ILK and downstream PI3-K/Akt and ERK1/2 pathways in PCO models of diabetic rabbit. Whereas, our study was compromised by several limitations. In the study, we did not place intraocular lens (IOL) following lens extraction, a larger space in the sac may influence the proliferation and migration of LECs by the absence of contact inhibition. Another limitation was that our preliminary data for the prevention of PCO were investigated in diabetic rabbit eyes. In order to apply echistatin to the human eyes during cataract surgery, more information for the expression pattern of integrin in the human lens should be needed, due to the life spans of rabbits is shorter compared with humans, and rabbit eyes have more proliferation activity than humans.

In conclusion, we found that echistatin could inhibit diabetic rabbit PCO occurrence and development after extracapsular lens extraction. These changes may be related to down-regulate the expression of ILK and inhibit its subsequent PI3-K/Akt and ERK1/2 signaling pathways. Echistatin had no significant side effects on other ocular tissues, such as corner and retina. Thus, our findings support that the echistatin can be a valuable tool for pharmacologic PCO or PCO with diabetic prophylaxis in the future.

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Disclosure of conflict of interest

None.

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