Matricellular Protein Levels in Aqueous Humor and Surgical Outcomes of Trabeculectomy

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In China, primary angle closure is estimated to cause unilateral blindness in 1.5 million individuals and bilateral blindness in another 1.5 million.1 Out of all forms of angle closure, acute primary angle closure (APAC) is regarded as an ophthalmic emergency, which is characterized by clinical signs and symptoms including rapid and excessive intraocular pressure (IOP) increase, corneal edema, a mid-dilated pupil, and nausea and/or vomiting.1 After the acute angle closure is broken, some APAC vascular congestion, eye pain, headache, nausea, and/or pressure (IOP) increase, corneal edema, a mid-dilated pupil, and symptoms including rapid and excessive intraocular ophthalmic emergency, which is characterized by clinical signs closure, acute primary angle closure (APAC) is regarded as an humor, leading to failure of the surgery.2 Excessive extracellular matrix (ECM) production and scar formation will close the generated passage for the aqueous humor, creating an artificial drainage route from the anterior chamber to the subconjunctival space, which can be defined as a surgical wound.2 Excessive extracellular matrix (ECM) production and scar formation will close the generated passage for the aqueous humor, leading to failure of the surgery.3

Glaucoma filtration surgery attempts to control IOP by creating an artificial drainage route from the anterior chamber to the subconjunctival space, which can be defined as a surgical wound.3 Excessive extracellular matrix (ECM) production and scar formation will close the generated passage for the aqueous humor, leading to failure of the surgery.4

In a previous study, we quantified levels of three matricellular proteins in the aqueous humor in patients with previous acute primary angle closure (APAC) and investigate their correlation with bleb morphology and surgical outcomes of trabeculectomy.

METHODS

Patients

This study was approved by the ethics committee of Shanghai General Hospital. Written informed consent was obtained from each patient, and all procedures were performed in accordance with the tenets of the Declaration of Helsinki. Included in this study were 40 eyes of 40 patients (18 males and 22 females; mean age, 62.5 ± 10.3 years) who underwent trabeculectomy for uncontrolled IOP due to APAC for at least 6 months. Of these patients, 25 were previously treated for APAC in the contralateral eye, whereas the other 15 were previously blind in the contralateral eye. The surgical outcomes were compared with those of 35 control patients (19 males and 16 females; mean age, 62.3 ± 10.0 years) who underwent trabeculectomy for glaucomatous cataract.

RESULTS

When previous APAC eyes were divided according to surgical outcome 18 months after trabeculectomy, SPARC protein was significantly higher in aqueous humor in the failure group (P < 0.009). When previous APAC eyes were divided according to SPARC level, eyes with low SPARC levels had significantly higher overall success rate compared with eyes with high SPARC levels (P = 0.005 for complete success and P = 0.018 for qualified success). Multiple logistic regression analyses showed that eyes with higher levels of SPARC were more likely to have a failed filtration surgery than were eyes with lower levels of SPARC. For complete success, P = 0.006 and odds ratio (OR) = 6.458; for qualified success, P = 0.033 and OR = 2.608. The level of SPARC was found to have a positive correlation with bleb score (P < 0.001, R2 = 0.471).

CONCLUSIONS

In previous APAC patients, the SPARC level in aqueous humor is a prognostic factor for surgical results of trabeculectomy. Modulation of SPARC expression may have potential clinical applications after filtration surgery.

Keywords: matricellular proteins, SPARC, trabeculectomy

PURPOSE. The purpose of this study was to quantify levels of three matricellular proteins in the aqueous humor in patients with previous acute primary angle closure (APAC) and investigate their correlation with bleb morphology and surgical outcomes of trabeculectomy.

METHODS. In this prospective study, aqueous humor samples were collected from 40 previous APAC eyes. Concentrations of three matricellular proteins—secreted protein acidic and rich in cysteine (SPARC), thrombospondin-2, and osteopontin—were measured using multiplexed immunoassays kits. Intraocular pressure was measured using Goldmann application tonometry. Bleb morphology was assessed using anterior segment optical coherence tomography, and bleb score was calculated according to bleb size and reflectivity.

RESULTS. When previous APAC eyes were divided according to surgical outcome 18 months after trabeculectomy, SPARC protein was significantly higher in aqueous humor in the failure group (P < 0.009). When previous APAC eyes were divided according to SPARC level, eyes with low SPARC levels had significantly higher overall success rate compared with eyes with high SPARC levels (P = 0.005 for complete success and P = 0.018 for qualified success). Multiple logistic regression analyses showed that eyes with higher levels of SPARC were more likely to have a failed filtration surgery than were eyes with lower levels of SPARC. For complete success, P = 0.006 and odds ratio (OR) = 6.458; for qualified success, P = 0.033 and OR = 2.608. The level of SPARC was found to have a positive correlation with bleb score (P < 0.001, R2 = 0.471).

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In China, primary angle closure is estimated to cause unilateral blindness in 1.5 million individuals and bilateral blindness in another 1.5 million.1 Out of all forms of angle closure, acute primary angle closure (APAC) is regarded as an ophthalmic emergency, which is characterized by clinical signs and symptoms including rapid and excessive intraocular pressure (IOP) increase, corneal edema, a mid-dilated pupil, vascular congestion, eye pain, headache, nausea, and/or vomiting.1 After the acute angle closure is broken, some APAC patients will still have considerable peripheral anterior synechiae and uncontrolled IOP, and filtration surgery will then be performed.

Glaucoma filtration surgery attempts to control IOP by creating an artificial drainage route from the anterior chamber to the subconjunctival space, which can be defined as a surgical wound.2 Excessive extracellular matrix (ECM) production and scar formation will close the generated passage for the aqueous humor, leading to failure of the surgery.3

In a previous study, we quantified levels of matricellular proteins in aqueous humor samples from APAC and non-glaucomatous cataract eyes. Increased levels of secreted protein acidic and rich in cysteine (SPARC), thrombospondin-2 (TSP-2), and osteopontin have been detected in APAC eyes compared with cataract eyes.4 Matricellular proteins represent an intriguing family of nonstructural proteins. Over the past 5 years, significant strides have been made in our understanding that those proteins play roles in the promotion of ECM deposition and subsequently occlusion of the outflow pathway.5 Therefore, their elevation in aqueous humor may have a great influence on the outcome and prognosis of filtration surgery. However, to date, their correlation with surgical outcomes in APAC has not been described. In this present study, we investigated the levels of SPARC, TSP-2, and osteopontin in the aqueous humor at the time of trabeculectomy in patients with APAC. The surgical outcomes after 18 months were also analyzed.

METHODS

Patients

This study was approved by the ethics committee of Shanghai General Hospital. Written informed consent was obtained from each patient, and all procedures were performed in accordance with the tenets of the Declaration of Helsinki.
with the Declaration of Helsinki. Subjects included were previous APAC patients who had had an APAC attack within 1 month before admission to our hospital. Patients still had uncontrolled IOP under standardized antiglaucomatous medications after pupil block was released. All patients agreed to receive trabeculectomy to reduce IOP. Participants were recruited prospectively and consecutively at Shanghai General Hospital from May 2016 to November 2016. Exclusion criteria were (1) eyes with ocular diseases other than APAC, (2) eyes with a history of intraocular incisional surgery, and (3) eyes that had an IOP value of <21 mm Hg at all three visits prior to surgery. The standard medication treatment for previous APAC is listed below. Some or all of the following were used according to the patient’s IOP and physical status: (1) topical pilocarpine 1% four times daily, (2) topical β-adrenergic antagonists two times daily, (3) topical α2-adrenergic agonists two times daily, (3) topical steroids four times daily, and (4) topical and/or oral carbonic anhydrase inhibitors.

**Surgical Technique and Postoperative Care**

Trabeculectomy was performed by one surgeon (MF). After making a fornix-based conjunctival flap and dissecting a limbus-based 4*4-mm scleral flap, a 5-fluorouracil-soaked (25 mg/mL) sponge was placed underneath the conjunctival flap for 3 minutes. Afterward, 250 mL balanced salt solution was used to wash the surgical area. A trabeculectomy and a basal iridectomy were then performed. The scleral flap was sutured with two 10-0 nylon sutures at its corners, and the centers of two sides were closed with two adjustable sutures to allow minimal leakage during reformation of the anterior chamber. Finally, the conjunctiva was closed with 10-0 nylon suture.

A topical antibiotic and a topical nonsteroidal anti-inflammatory medication were given four times daily for 1 week. Topical corticosteroids were used every 2 to 3 hours for the first week, and then tapered over the next 1 to 2 months. No antimetabolites were used during follow-up. In case of postoperative IOP measurements > 21 mm Hg, despite ocular massage and loosening of adjustable sutures, IOP-lowering medication was added. In case of still inadequate IOP control, additional surgical procedures could be performed as required.

**Collection of Aqueous Humor**

Briefly, aqueous humor samples (50–100 µL) were collected at the very beginning of trabeculectomy through limbal paracentesis. Samples were immediately stored in liquid nitrogen and transferred to −80°C until they were analyzed.

**Measurement of Cytokine Levels in Aqueous Humor**

Three matricellular proteins, SPARC, TSP-2, and osteopontin, were detected using an assay (Luminex Screening Human Magnetic Assay; R&D Systems, Inc., Minneapolis, MN, USA). The assay was performed according to the manufacturer’s instructions. Aqueous humor samples were diluted 1:2 for the assay, and 50 µL of the diluted samples was added to each well. Fluorescence intensity was acquired and analyzed using software (Luminex xponent3.1; Luminex, Austin, TX, USA).

**Outcome Measures**

The preoperative IOP and all topical antiglaucomatous medications used were recorded before surgery. Patients were followed up at 1 week and 1, 3, 6, 12, and 18 months. Goldmann applanation tonometry and slit-lamp biomicroscopy were performed at each postoperative visit. The number of antiglaucomatous medications used and any additional surgeries performed were also recorded. We defined surgical failure as an IOP value ≥ 21 mm Hg or if any additional glaucoma surgeries were required. If IOP of one visit could be controlled lower than 21 mm Hg without the use of topical antiglaucomatous medication, the visit was defined as “complete success.” If IOP of one visit was < 21 mm Hg with eye drops, the visit was defined as a “qualified success.” For instance, a patient who had an IOP (without eye drops) of 24 mm Hg on one follow-up and 18 mm Hg (with eye drops) at the subsequent visit was defined as “qualified success” at the second visit.

**Statistical Analysis**

Commercially available statistical analysis software (SPSS version 22.0; SPSS, Inc., Chicago, IL, USA) was used for the analyses. Continuous variables (e.g., levels of matricellular proteins) were expressed as mean and standard deviation. Categorical variables (e.g., sex) were expressed as number and frequency. Independent Student’s t-tests were applied to compare data between two groups. Pearson χ² test was used for categorical data. Binary logistic regressions were used to assess the relationship between the clinical outcome and matricellular protein levels in the aqueous humor. If any matricellular protein was detected to be a significant risk factor for trabeculectomy failure, we divided the patients into two groups according to the level of this protein, and Kaplan Meyer survival curves were used to display the success rate of end points over time between groups. Linear regressions were used to assess the relationship between the matricellular protein levels and bleb score assessed by AS-OCT. The coefficient of determination R² was used to express the proportion of the variation in the dependent variable explained by the regression model. The level of statistical significance was set at P < 0.05.

**RESULTS**

**Patients**

In total, 40 previous APAC patients were included in this study. The clinical characteristics are shown in Table 1. The mean (SD) levels (ng/mL) of SPARC, TSP-2, and osteopontin in aqueous humor samples were 2.938 (1.159), 0.245 (0.408), and 18.382 (14.412), respectively. All patients finished the 18 months of follow-up. During the follow-up period, 29 eyes (72.5%) were classified into the complete success group and 11 eyes (27.5%) into the failure group. There were no
significant differences between both groups considering age, sex, preoperative IOP, numbers of preoperative medications, and the interval between the onset of APAC and trabeculectomy. However, the mean levels of SPARC and osteopontin were significantly higher in the 11 failed eyes compared to the 29 complete successful eyes ($P = 0.009$ and 0.004, respectively) (Table 1).

**Correlation Between Matricellular Proteins and Surgical Results**

The preoperative IOP and levels of SPARC, TSP-2, and osteopontin were included in the multiple logistic regression analysis. Considering the matricellular protein SPARC, eyes with higher levels of SPARC were more likely to have a failed filtration surgery than were eyes with lower levels of SPARC. For complete success, $P = 0.006$, odds ratio (OR) = 6.458, and 95% confidence interval (CI) = 1.728 to 24.133; for qualified success, $P = 0.033$, OR = 2.608, and 95% CI = 1.081 to 6.292. The other factors, including preoperative IOP and levels of TSP-2 and osteopontin, were not associated with the outcome of trabeculectomy. Results indicated that high SPARC level was a significant risk factor for trabeculectomy failure.

We subsequently divided the 40 previous APAC patients into two groups according to the level of SPARC. Twenty eyes with a SPARC level lower than the median value (<2.638 ng/mL) were included in group L, and 20 patients with SPARC level higher than the median value (>2.638 ng/mL) were included in group H. The postoperative outcomes including postoperative IOP, antiglaucomatous eye drop usage, and success rates are summarized in Table 2. The complete success rates at 18 months after trabeculectomy were 90% and 55% in group L and group H, respectively. The qualified success rates at 18 months after trabeculectomy were 100% and 75% in group L and group H, respectively. A Wilcoxon test revealed that the differences in success rates at 18 months between both groups were significant ($P = 0.013$ for complete success, and $P = 0.047$ for qualified success). The Kaplan-Meier survival plots were drawn to investigate the overall success rate between both groups (Fig. 1). Eyes with low SPARC levels (group L) had significantly higher complete success rate and qualified success rate over the 18 months of follow-up compared to eyes with high SPARC levels (group H) ($P = 0.005$ and $P = 0.018$, respectively) (Fig. 1).

**Correlation Between Matricellular Proteins and Bleb Scores**

The relationship between levels of SPARC, TSP-2, and osteopontin with bleb scores at postoperative18 months was evaluated (Fig. 2). Only the SPARC level had significantly positive correlation with bleb score ($P < 0.001, R^2 = 0.471$), for which the bleb score ($y$) was related to the SPARC level ($x$) according to the formula $y = 0.545 + 0.408x$.

**DISCUSSION**

Trabeculectomy is the gold standard in surgical management of medically uncontrolled glaucoma. The poor outcome is usually due to the fibrosis of the surgical site. One possible explanation for the enhanced fibrosis is the overexpression of related

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Previous APAC With Low Levels of SPARC, Group A</th>
<th>Previous APAC With High Levels of SPARC, Group B</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no.</td>
<td>20</td>
<td>20</td>
<td>$&lt;0.001$</td>
</tr>
<tr>
<td>SPARC level, ng/mL</td>
<td>$2.148 \pm 0.379$</td>
<td>$3.727 \pm 1.141$</td>
<td></td>
</tr>
<tr>
<td>Probability of complete success, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6 months</td>
<td>95</td>
<td>75</td>
<td>0.182</td>
</tr>
<tr>
<td>12 months</td>
<td>90</td>
<td>65</td>
<td>0.127</td>
</tr>
<tr>
<td>18 months</td>
<td>90</td>
<td>55</td>
<td>0.013*</td>
</tr>
<tr>
<td>Probability of qualified success, %</td>
<td></td>
<td></td>
<td></td>
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<td>6 months</td>
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<td>75</td>
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<tr>
<td>IOP at 18 months postoperatively</td>
<td>$13.9 \pm 2.5$</td>
<td>$15.2 \pm 2.4$</td>
<td>0.115</td>
</tr>
<tr>
<td>Number of glaucoma eyedrops at 18 months postoperatively</td>
<td>$0.1 \pm 0.3 (0-1)$</td>
<td>$0.4 \pm 0.8 (0-2)$</td>
<td>0.195</td>
</tr>
</tbody>
</table>

* $P < 0.05$.  

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**TABLE 1.** Characteristics of 40 Previous APAC Patients Included

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Previous APAC</th>
<th>Successful Group</th>
<th>Failed Group</th>
<th>$P$ Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, no.</td>
<td>40</td>
<td>29</td>
<td>11</td>
<td>0.235</td>
</tr>
<tr>
<td>Male/female</td>
<td>15/25</td>
<td>13/16</td>
<td>2/9</td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>$68 \pm 11$</td>
<td>$69 \pm 10$</td>
<td>$66 \pm 13$</td>
<td>0.454</td>
</tr>
<tr>
<td>Preoperative IOP</td>
<td>$33.1 \pm 9.0$</td>
<td>$31.5 \pm 8.1$</td>
<td>$37.4 \pm 10.2$</td>
<td>0.066</td>
</tr>
<tr>
<td>Number of antiglaucoma eyedrops</td>
<td>$2.3 \pm 1.1$</td>
<td>$2.3 \pm 1.1$</td>
<td>$2.3 \pm 1.1$</td>
<td>0.094</td>
</tr>
<tr>
<td>Interval between the onset of APAC and trabeculectomy, d</td>
<td>$15.7 \pm 9.1$</td>
<td>$15.2 \pm 9.0$</td>
<td>$17.0 \pm 9.6$</td>
<td>0.575</td>
</tr>
<tr>
<td>SPARC, ng/mL</td>
<td>$2.98 \pm 1.159$</td>
<td>$2.525 \pm 0.639$</td>
<td>$4.025 \pm 1.517$</td>
<td>0.009*</td>
</tr>
<tr>
<td>Osteopontin, ng/mL</td>
<td>$0.245 \pm 0.408$</td>
<td>$0.146 \pm 0.225$</td>
<td>$0.505 \pm 0.636$</td>
<td>0.094</td>
</tr>
<tr>
<td>Osteopontin, ng/mL</td>
<td>$18.382$</td>
<td>$18.439 \pm 11.720$</td>
<td>$28.777 \pm 16.276$</td>
<td>0.004*</td>
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* $P < 0.01$.  

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**TABLE 2.** Surgical Results Between Groups With Low Levels of SPARC and High Levels of SPARC

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Cytokines, resulting in the disappearance of a functional filtration pathway. Fibrosis is regulated by a series of factors, of which the transforming growth factor-β (TGF-β) family has drawn the most attention. However, the CAT1520120 Trabeculectomy Study Group used a monoclonal antibody to TGF-β2 to prevent scarring after the first trabeculectomy, and they showed no difference between treatment and placebo groups in preventing the failure of primary trabeculectomy.14 It is conceivable that directing against TGF-β2 is too narrow an approach in humans. Other factors besides TGF-β2 are also involved, as are other factors that may mediate the effects of TGF and scar formation.

Matricellular proteins were first proposed in 1995 to describe a group of nonstructural secreted glycoproteins that enable cells to communicate with and control their surrounding ECM.15 The original family includes SPARC, TSP-1 and -2, tenascin-C and -X, osteopontin, and other proteins.14 They were found widely expressed in all kinds of glaucomatous eyes, and the overexpression of these proteins was noted to add ECM turnover.5,15,16 Our previous study showed that the levels of matricellular proteins, including SPARC, TSP-2, and osteopontin, were significantly elevated in eyes that suffered from acute angle closure.4 This elevation might lead to the ECM remodeling and subsequent outflow pathway occlusion. Therefore, in this study, we further investigated the correlation between these three proteins and surgical outcomes. A previous study17 suggests that trabeculectomy may have a lower risk of failure if performed later rather than earlier after an acute glaucoma attack. Thus, we recruited only patients who had an APAC attack within 1 month before admission to our hospital. We also analyzed the mean intervals between the onset of APAC and trabeculectomy in the complete success group and failure group, and no significant difference was detected between the two groups. Our study showed that SPARC was significantly elevated in the aqueous humor of patients with failed glaucoma surgery. Furthermore, multivariate analyses revealed that SPARC strongly correlated with the surgical outcome, indicating that aqueous humor SPARC level is a significant risk factor for trabeculectomy failure. We subsequently divided the 40 eyes into two groups according to the level of SPARC. Higher probability of complete and qualified success was achieved in the group with a lower SPARC level.

SPARC, also known as osteonectin or BM-40, is a calcium-binding matricellular glycoprotein. Many prior studies indicated a strong association between elevated SPARC expression and tissue fibrosis.18,19 Seet et al.20 found that SPARC deficiency resulted in improved surgical survival in a novel mouse model of glaucoma filtration surgery. They also revealed that SPARC modulated the wound fibrosis by delaying cell migration, reducing collagen contractility, and lowering expressions of profibrotic genes.21 The filtering bleb, which arises from the surgical site of glaucoma filtration surgeries, is thought to be an important site in which to observe the excessive fibrosis and ECM remodeling.22 Thus, we used AS-OCT to assess the bleb size and internal reflectivity at postoperative month 18 and analyzed its relationship to matricellular protein levels. Our results demonstrated that higher SPARC levels in aqueous humor associated with an increasing AS-OCT bleb score (smaller bleb size and higher reflectivity). This reaffirmed that high SPARC levels might result in excessive tissue remodeling in the later postoperative period with increased resistance to outflow and lead to surgical failure. Future studies are needed to clarify the role that SPARC plays in the postoperative scarring.

Several limitations apply to this study. First, although the study was prospectively designed, the sample size of this study was relatively small. Second, the glaucoma patients we recruited were previous APAC patients, and further research should investigate the relationship between matricellular proteins and surgical outcomes in other glaucoma patients, such as in open angle glaucoma. Moreover, we only had AS-OCT data at postoperative month 18, and the grade system may not be descriptive enough compared to the traditional Moorfields Bleb Grading System and Indiana Bleb Appearance Grading Scale. However, we found this classification to be very practical and simplified, and our preliminary data did show that lower matricellular level associated with lower bleb score and better surgical outcome.

In summary, the levels of SPARC strongly correlated with outcome of filtration surgery. Targeting of SPARC expression...
Matricellular Proteins and Trabeculectomy Outcome

may have potential clinical applications in reducing postoperative scarring after glaucoma surgery.

Acknowledgments

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References