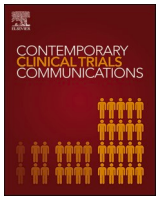
Contemporary Clinical Trials Communications 33 (2023) 101160

Contents lists available at ScienceDirect

Contemporary Clinical Trials Communications

journal homepage: www.elsevier.com/locate/conctc



A multicenter phase II study on the safety of rho-kinase inhibitor (ripasudil) with needling for the patients after trabeculectomy

Yu Mizuno [a,*], Kaori Komatsu [a], Kana Tokumo [a], Naoki Okada [a], Hiromitsu Onoe [a], Hideaki Okumichi [a], Kazuyuki Hirooka [a], Yukiko Miura [b], Yoshiaki Kiuchi [a]

[a] Department of Ophthalmology and Visual Science, Hiroshima University, 1-2-3 Kasumi Minamiku, Hiroshima, 734-8551, Japan
[b] Hiroshima Eye Clinic, 13-4, Noborimachi Nakaku, Hiroshima, 730-0016, Japan

ARTICLE INFO

Keywords:
Glaucoma
Trabeculectomy
Needling
A rho-associated protein kinase inhibitor
Ripasudil

ABSTRACT

Background: There is no established method of maintaining or reducing intra ocular pressure after the needling procedure for failing blebs post trabeculectomy. Regarding newer antihypertensive medications, ripasudil, which is a rho-associated protein kinase inhibitor ophthalmic solution, was able to prevent excessive scarring in vitro. This study aims to evaluate the safety of glaucoma patients submitted to the needling procedure and administered ripasudil for preventing scarring after the procedure. We also investigate the efficacy of ripasudil after needling for bleb failure through suppression of fibrosis to the bleb.

Methods: This study is a multicenter, open-label, single-arm, phase II trial to evaluate the safety and efficacy of ripasudil in glaucoma patients after the needling procedure. Forty patients who will undergo needling at least 3 months after trabeculectomy will be recruited in Hiroshima university hospital and Hiroshima eye clinic. All the patients will instill ripasudil two times per day for three months after the needling procedure. The primary endpoint is the safety of ripasudil.

Conclusions: We plan to establish the safety of ripasudil and to collect information involving the efficacy of ripasudil widely in this study.

1. Introduction

Glaucoma is an optic neuropathy characterized by gradual progressive morphological changes in the optic disc and visual field loss [1]. Trabeculectomy is an effective surgical technique for lowering the intraocular pressure (IOP) to slow the progression of visual field loss in glaucoma patients [2]. However, we often experience bleb failure due to fibrosis. Transconjunctival bleb needling procedure is designed to rebuild failing blebs by mechanically stripping the adhesions.

Regarding newer antihypertensive medications, ripasudil (Glanatec®, ophthalmic solution 0.4%, Kowa company, Ltd., Japan), a rho-associated protein kinase (ROCK) inhibitor ophthalmic solution was approved in Japan for the treatment of glaucoma or ocular hypertension since 2014. There are several reports that topical instillation treatment with ROCK inhibitor Y-27632 was able to prevent excessive scarring in vitro and after glaucoma filtration surgery in rabbit models [3,4]. However, there was no report for use/disuse of topical instillation treatment with ripasudil in the success after the needling procedure. We hypothesize that the use of ROCK inhibitor, ripasudil may prevent fibrosis after needling surgery as well as antimetabolites, such as mitomycin C (MMC) or 5-fluorouracil (5-FU) which might pre-vent fibrosis. Furthermore, eyedrop therapy is less invasive compared with the injection of antimetabolites in needling procedure.

In this study, we evaluate the safety of glaucoma patients submitted to the needling procedure without MMC and administered a ROCK inhibitor, ripasudil ophthalmic solution, after the procedure. We also investigate the efficacy of ROCK inhibitor after needling for bleb failure through suppression of fibrosis to the bleb.

2. Materials and methods

2.1. Study design

This study is a multicenter, open-label, single-arm, phase II trial to evaluate the safety and efficacy of ripasudil in glaucoma patients after the needling procedure without antimetabolites use. The study will be

* Corresponding author.
E-mail address: ymizuno@hiroshima-u.ac.jp (Y. Mizuno).

https://doi.org/10.1016/j.conctc.2023.101160
Received 24 March 2023; Received in revised form 26 May 2023; Accepted 3 June 2023
Available online 4 June 2023

conducted at Hiroshima university hospital and Hiroshima eye clinic (see Fig. 1).

2.2. Needling procedure

All needling procedures are performed in an outpatient department. All procedures are performed by the specialists of glaucoma who are listed in the protocol. After the administration of topical anesthesia (0.4% oxybuprocaine and 0.1% adrenaline), we apply iodine and polyvinyl alcohol (PA·IODO Ophthalmic and Eye washing solution diluted six times with saline solution) to the external eye. Subsequently, we inject 0.1 mL of 2% xylocaine with epinephrine using a 27-gage needle at the subconjunctiva approximately 10 mm distal to the bleb. The needle is introduced under the scleral flap, and the fibrotic tissues are then cut and raised. After the procedure, all patients are instructed to use topical antibiotics (1.5% levofloxacin) and anti-inflammatory (0.1% fluorometholone ophthalmic suspension) eye drops four times daily.

2.3. Eligibility criteria

After obtaining informed consent from patients, potential participants will be screened by investigators. The inclusion and exclusion criteria are as follows.

2.3.1. Inclusion criteria

The study will enroll those who confirm all of the following criteria.

(1) Patients undergoing needling at least 3 months after trabeculectomy.
(2) Patients must be at least 20 years of age at the time of consent.
(3) Patients who can make outpatient visits for 3 months after needling.
(4) Patients who can provide consent to participate in this study.

2.3.2. Exclusion criteria

Patients having any of the following criteria are not eligible for this study.

(1) Pregnant, lactating, or possibly pregnant women.
(2) Patients in whom it is difficult to measure the IOP with a Goldmann applanation tonometer.
(3) Patients with allergy to ripasudil.
(4) Patients less than 3 months post-internal eye surgery (except for cataract surgery performed at the same time with trabeculectomy).
(5) Patients with a history of conjunctival surgery (except trabeculectomy).
(6) Patients who received eye drops or oral medication containing tranilast at least 30 days prior to the start of the study.
(7) Patients who received ripasudil at least 3 days prior to the start of the study
(8) Patients who are deemed to be inappropriate by the principal investigator/approving ophthalmologist to participate in this study.
(9) Patients who are participating or intend to participate in other clinical studies while participating in this study.

2.4. Endpoints

The primary endpoint of the study is the safety of ripasudil in patients who received it immediately after the needling procedure. Ophthalmic and systemic examinations will be performed to examine the incidence of local and systemic adverse events (AEs) and adverse drug reactions (ADRs). An AE will be registered even if the abnormality occurs in the eye opposite to that in which ripasudil ophthalmic solution was instilled.

The secondary endpoints are the efficacy of ripasudil as follows.

(1) Evaluate each glaucoma type and the incidence of ocular (whether one or both eyes) AEs and ADRs.
(2) Pre-treatment and medications used for ocular and systemic diseases and incidence of ocular (whether one or both eyes) AEs and ADRs.
(3) Survival rate at 3 months after the needling procedure without using MMC.
1) We define absolute success as a >20% reduction in IOP from pre-needling without using antihypertensive medications (excluding the instillation of ripasudil). When the IOP is higher than the criteria for two consecutive measurements, it is considered to have failed at the first time point when the IOP was above the criteria. Need for re-needling or another glaucoma surgery was classified as failures.
2) Relative success is defined as an IOP of (A) 4 mmHg or more but less than 22 mmHg, (B) 4 mmHg or more but less than 19 mmHg, (C) 4 mmHg or more but less than 16 mmHg, or (D) 4 mmHg or more but less than 13 mmHg. When the IOP was higher than the

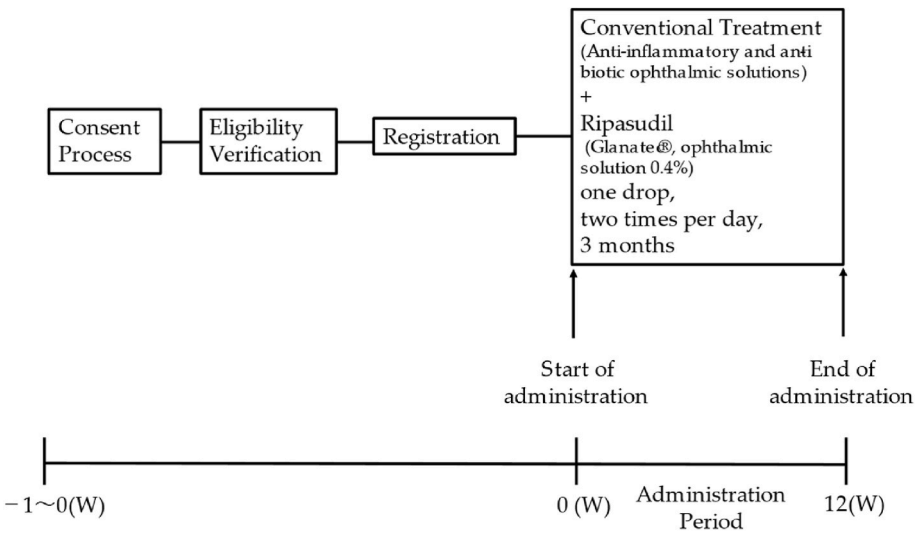


Fig. 1. Study flow diagram.

criteria for two consecutive measurements, it was considered to have failed at the first time point when IOP was above the criteria. Need for re-needling or another glaucoma surgery were classified as failures.

(4) Evaluate changes in IOP, aqueous flare, corneal thickness, corneal endothelial cell density, bleb score [5], anterior-segment optical coherence tomography (OCT) for the bleb, contents of antihypertensive medications, and Humphry field analyzer at 1 week, 1 month, and 3 months before and after needling.

(5) Evaluate changes in IOP, aqueous flare, corneal thickness, corneal endothelial cell density, bleb score, anterior-segment OCT for the bleb, contents of antihypertensive medications, and Humphry field analyzer at 1 week, 1 month, and 3 months before and after needling for each glaucoma type.

2.5. Investigational drug

Glanatec ophthalmic solution 0.4% (Kowa Co., Japan) containing ripasudil hydrochloride hydrate will be used for this trial. Glanatec ophthalmic solution 0.4% was granted marketing approval for the treatment of glaucoma and ocular hypertension in Japan since 2014. The investigational drug and its safety information have been obtained from Kowa Co.

2.6. Intervention

The study schedule for the patients is shown in Table 1. A total of 40 patients participating in the study will instill one drop of ripasudil ophthalmic solution in the eye after needling without using MMC, two times per day for 3 months. The safety and efficacy of ripasudil will be assessed at the end of the 3 months treatment period.

2.7. AE and ADR recording

The investigators will collect and record all AEs and ADRs that occur in patients during the study period. Serious AEs (SAEs) will be defined as follows; (1) death of the patients, (2) the occurrence of AEs that may lead to death, (3) permanent disability of the patients, (4) the occurrence of AEs that may lead to permanent disability of the patients, and (5) the occurrence of AEs that require hospitalization. All related SAEs will be summarized and reported to the ethics committee.

2.8. Rescue treatment

When the patients need additional IOP control in the study period, there are no re-strictions on the rescue treatment.

2.9. Safety evaluation

The incidence of AEs and ADRs will be evaluated in the study period.

2.10. Data collection and management

2.10.1. Visual acuity

Visual acuity (VA) and best-corrected visual acuity (BCVA) will be measured before any techniques that could affect vision. VA and BCVA will be performed using Landolt C with standard illumination. The score will be recorded on a data form.

2.10.2. IOP

We will use Goldman Applanation Tonometer (Haag-Streit, Köniz, Switzerland) to measure the IOP. In all patients, IOP will be measured three times, and the mean of the three measurements will be used for assessment.

2.10.3. Fundus examination

The optic disc will be assessed by stereo photographs using fundus cameras (eidon, CenterVue S.p.A., Italy, or nonmyd wx, Kowa, Japan).

2.10.4. Aqueous flare

Aqueous flare will be measured without pupil dilatation using laser flare cell mater (FM-600, Kowa, Japan). All the patients will be measured three times, and the average score will be recorded.

2.10.5. Corneal endothelial cell density (ECD)

Corneal endothelial cell density will be counted using a non-contact type specular microscope (EM-4000, TOMEY, JAPAN or SP-3000P, TOPCON, Japan). The captured images will be 0.50 mm high and 0.25 mm wide. We will study the central point, and the score will be recorded on a data form.

2.10.6. Bleb score

For classifying the morphological slit lamp appearance of the blebs, the Indiana Bleb Appearance Grading Scale, which was formed from height (H0–H3), extent (E0–E3), vascularity (V0–V4), and leakage graded with the Seidel test (S0–S2), will be evaluated.

2.10.7. Anterior-segment OCT for the bleb

Anterior-segment OCT images of the blebs will be evaluated using CASIA 2 (Tomey Corp, Japan).

Table 1
Study schedule.

Timing (D = day, W = week)	Entry	Needling procedure	Study period				Cassation
		Day 0	Day 1	1 W	4 W	12 W	
Entry	○						
Informed consent	○						
Eligibility screen	○						
Registration	○						
Demographic data and medical history	○						
Adverse events and adverse drug reactions			○	○	○	○	○
Optometry and slit-lamp examination	○			○	○	○	○
Visual acuity	○				○	○	○
Intraocular pressure	○			○	○	○	○
Fundus examination	○			○	○	○	○
Aqueous flare	○			○	○	○	○
Corneal endothelial cell density	○			○	○	○	○
Bleb score	○			○	○	○	○
Anterior-segment optical coherence tomography for the bleb	○			○	○	○	○
Contents of antihypertensive medications	○			○	○	○	○
Humphry field analyzer	○				○	○	○
Pregnancy test	○						

2.10.8. Contents of antihypertensive medications

The contents of antihypertensive medications will be administrated at all visits.

2.10.9. Visual field testing

All patients will be performed with Humphrey field analyzer Mark 3 (Carl Zeiss Meditec, Dublin, CA, USA) and the Swedish interactive threshold algorithm standard 24-2 program.

2.10.10. Pregnancy test

Women of childbearing age will need to undergo a urine pregnancy test at the first visit. Ripasudil will be instilled after the results are confirmed negative.

2.11. Data management

All the original data collected will be stored in the electronic data capture (EDC) system. The EDC system is secured digitally on a password-protected internet server. The data will be directly entered into the EDC system by investigators who belong to the study team. Any changes from the raw data will be documented in the EDC system.

2.12. Sample size

A total of 40 eyes from 40 patients will be enrolled in this study. The sample size is estimated based on past medical records. The number of patients per year at Hiroshima university hospital is approximately 80, and it is the same at Hiroshima eye clinic. Considering the exclusion criteria and participation rate in past clinical trials, the expected rate of consent acquisition is approximately 60%. It will take approximately 3–6 months to accumulate 40 patients. Since this study is an exploratory study, the target number of patients was set in consideration of the feasibility to obtain the minimum information necessary for the next study.

2.13. Statistical analysis

The following items will be collected as demographic data: sex, age, type of glaucoma, content of antihypertensive medications, history.

The primary endpoint, which is the safety of ripasudil in patients who received it immediately after the needling procedure, will be calculated as the number and per-centage of cases with AEs and ADRs. All patients who received at least one time of ripasudil will be included in the analysis population and counted by the type of AEs or ADRs.

The secondary endpoint, which will evaluate the efficacy of ripasudil, will be analyzed. The analysis set are defined as Full Analysis Set (FAS) and Per Protocol Set (PPS). The FAS is defined as the population of all study subjects excluding the following cases that do not meet eligibility criteria. The PPS is defined as the population of FAS excluding the following research subjects: patients with no available measures of the primary endpoints and patients with serious violations of the study protocol. We will use FAS as the analysis set and PPS as the sub-analysis set for each of the secondary endpoints. Survival rate will be estimated by the Kaplan–Meier method. The differences between glaucoma type, bleb score, or time period before and after the needling procedure will be analyzed using the Student's t-test (or Wilcoxon's rank sum test).

2.14. Patient and public involvement

Patients and/or the public were not involved in the design of this study.

2.15. Ethics and dissemination

This study will be performed according to the ethical principles from the Declaration of Helsinki and the Good Clinical Practice guidelines. This study was approved by the institutional review board. All participants will be informed fully on the purpose and methods of the study. Consent forms will be signed from the participants with the right to withdraw at any time. Confidentiality of the participants will be respected, even for collecting data by investigators. We will present the results of this trial in international peer-reviewed journals and at international and national conferences. Approval for this study has been obtained from the Hiroshima University Certified Review Board (Approval No. CRB210008, May 26, 2022).

3. Discussion

The primary aim of this study is to establish the safety of ripasudil and to collect in-formation regarding the efficacy of ripasudil widely for conducting phase III trials.

Seven-day repeated instillation of ripasudil in a phase I clinical trial revealed that ripasudil showed tolerable AEs, such as slight to moderate conjunctival hyperemia in more than half of the healthy volunteers, and it was resolved within 90 min after instillation [6]. The safety of ripasudil was similar to that of patients with open-angle glaucoma or ocular hypertension [7,8].

In the post marketing survey, the incidence of conjunctival hyperemia was 4.0% (122/3058), and total of ADRs were 8.0% (244/3058) in three months. These results indicate that the incidence of AEs or ADRs is not expected to be high in our study within periods of three months. However, there are few reports on the use of ripasudil in the early period after filtration surgeries including the needling procedure, and there is a risk of ocular hypotension or other ADRs. Therefore, we aim to evaluate the safety of ripasudil in the study.

IOP reduction is the most effective treatment for glaucoma patients [9,10]. Trabeculectomy is the gold standard surgical technique for lowering the IOP [2]. It creates a drainage pathway between the anterior chamber and sub-Tenon's space to lead a subconjunctival space for the aqueous humor, referred as a filtrating bleb. The success of trabeculectomy depends on the continuous passage of aqueous humor between the anterior chamber and subconjunctival space.

However, the most common case of failure is with fibroblast proliferation and scar formation under the conjunctival and episcleral interface of the filtrating bleb [11,12]. There are several treatments available for bleb failure after trabeculectomy. These include antihypertensive medications, surgical treatment including the needling procedure with or without antimetabolites use (5-FU or MMC) [13–15], or needle revision [16] with a variety of techniques. At present, there is no gold standard treatment for bleb failure, and different glaucoma specialists may change the details of practices according to their own experience [17]. Transconjunctival bleb needling procedure is designed to rebuild the failing blebs by mechanically stripping the adhesions. To prevent fibroblast proliferation, antimetabolites, such as 5-FU or MMC, have become widely accepted in bleb needling as for subconjunctival injection [18–20]. There are several studies [20–22] in which the use of antimetabolites in the needling procedure was considered to have led to IOP control or reduced antihypertensive medications. However, several reports revealed that there was no apparent difference in IOP reduction between antimetabolites (MMC vs. 5-FU) [23–25]. Interestingly, there is a report that the use of antimetabolites or not did not show a statistical difference in reducing IOP [26]. Hence, either the use of MMC or 5-FU, or even the use of antimetabolites, which contributes to achieve IOP control and reduce antihypertensive medications, was not evaluated [27].

In glaucoma filtration surgery, scleral flap or conjunctiva was exposed to cytokines and growth factors produced by various types of inflammatory cells and fibroblasts [28]. These responses provoke the activation and migration of fibroblasts and other inflammatory cells. Activated fibroblasts produce and secrete extracellular matrix such as collagen for fibrosis. ROCK inhibitor has been reported to suppress the expression of extracellular matrix degrading enzymes in fibroblasts and

to decrease extracellular matrix secretion [29]. Diah Gemala Ibrahim et al. revealed that ROCK inhibitor Y-27632 may inhibit fibrosis and improve outcomes after glaucoma filtration surgery through inhibition of transdifferentiation of Tenon fibroblasts into myofibroblasts as well as transforming growth factor β(TGF-β) and Mitogen-activated Protein Kinase (MAPK) signaling after surgery [3]. Honjyo M. et al. also reported that topical instillation of ROCK inhibitor Y-27632 inhibited wound healing and fibroproliferation after filtration surgery in a rabbit model [4]. However, there is no study in which ROCK inhibitor, ripasudil, was instilled for the prevention of fibrosis after the needling procedure, which is a less invasive procedure than subconjunctival inject of antimetabolites in the needling procedure.

Thus, we plan to establish the safety of ripasudil and to collect information involving the efficacy of ripasudil widely in this study.

If we confirm the safety and efficacy of ripasudil for instillation after needling in this study, we will continue to prepare for a phase III trial to evaluate less invasive strategies after the needling procedure through maintaining the shape of the bleb to maintain or reduce IOP for preventing progression of visual field loss. In parallel with a phase III trial, we also plan to conduct an observational study to verify use of antimetabolites (MMC or 5-FU) or ripasudil (without the use of antimetabolites) using a comparison-matched group.

There is a several limitations in the protocol. First, because of the lack of dose response test, including optical frequency of ripasudil or optical ripasudil concentration. However, Ripasudil, Glanatec®, was approved in Japan, as an ophthalmic solution at a concentration of 0.4% and two times per day for instillation. We believe at least the use of the approved range is one of the indicators for the safety in the patients. Depending on the results of this study, we may need to plan additional study for optical frequency of ripasudil or optical ripasudil concentration as a phase IIb trial (including placebo) before a phase III trial not only for the safety but also for the efficacy. Second, the study is designed as a single-arm, because we defined the primary end point for the safety of ripasudil in the patients. Also, we collect the efficacy of ripasudil (IOP, aqueous flare, and so on) as the secondary endpoints. Therefore, the efficacy will be a comparison of the patients before and after needling, so especially in terms of the efficacy of ripasudil in patients after the needling does not adequately rule out all potential or on the actual course of the disease other than those arising from the pharmacologic action of ripasudil than the placebo control design. Also, though we plan to evaluate changes in IOP, bleb score, and anti-segment OCT images for the blebs, we need to consider that these evaluations is not only due to the anti-scarring effect of ripasudil rather than to its ability to lower the IOP, by increasing conventional aqueous flow. However, there are no previous reports on the clinical use of ripasudil after the needling without using MMC, it is difficult to define in advance the obvious criteria of efficacy in this study. So that, we plan to use the results of the efficacy of the study as a reference for the next study as well as compared with the previous report without the use of antimetabolites in the needling despite the limitation of not being able to completely control bias [22].

4. Conclusions

In this study, we aim to evaluate a less invasive treatment after the needling procedure, since treatments to maintain or reduce intra ocular pressure after the needling procedure have not been evaluated.

Author contributions

Y.M. is the principal investigator of this trial and wrote the manuscript with critical assistance from K.K., N.K., H.O., K.T., H.O., K.H., Y. M., and Y.K. Authors Y.M. and Y.K. conceived and designed the trial protocol. Y.M., N.K., H.O., K.T., H.O., K.H., Y.M., and Y.K. collected the clinical data. K.K. and Y.K. supported this project from a regulatory perspective. All authors contributed to multiple revisions of the trial protocol and approved the final manuscript version for publication. Y.M. is responsible for the statistical analyses in this study.

Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Hiroshima University Certified Review Board (Approval No. CRB210008, May 26, 2022).

Informed consent statement

All participants will be made fully aware of the aims of the research and written informed consent will be obtained from all subjects.

Data availability statement

Not applicable.

Funding statement

This research was supported by Hiroshima University Hospital.

Trial registration

The trial was registered at jRCT on June 9th, 2022 (jRCTs061220032, https://jrct.niph.go.jp/latest-detail/jRCTs061220032).

Trial status

This trial is currently recruiting patients. The trial is expected to end on September 30th, 2023. The protocol is Version 1.2 dated October 3rd, 2022.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

None.

References

[1] H.A. Quigley, E.M. Addicks, W.R. Green, et al., Optic nerve damage in human glaucoma, Arch. Ophthalmol. 99 (1981) 635–649.
[2] J.E. Cairns, Trabeculectomy. Preliminary report of a new method, Am. J. Ophthalmol. 66 (1968) 673–679.
[3] D.G. Ibrahim, J. Ko, W. Iwata, et al., An in vitro study of scarring formation mediated by human Tenon fibroblasts: effect of Y-27632, a Rho kinase inhibitor, Cell Biochem. Funct. 37 (2019) 113–124.
[4] M. Honjo, H. Tanihara, T. Kameda, et al., Potential role of rho-associated protein kinase inhibitor Y-27632 in glaucoma filtration surgery, Invest. Ophthalmol. Vis. Sci. 48 (2007) 5549–5557.
[5] L.B. Cantor, A. Mantravadi, D. WuDunn, et al., Morphologic classification of filtering blebs after glaucoma filtration surgery: the Indiana bleb appearance grading scale, J. Glaucoma 12 (2003) 266–271.
[6] T. Hidenobu, I. Toshihiro, Y. Tetsuya, et al., Phase 1 clinical trials of a selective Rho kinase inhibitor, K-115, JAMA Ophthalmol 131 (2013) 1288–1295.
[7] T. Hidenobu, I. Toshihiro, Y. Tetsuya, et al., Intra-ocular pressure-lowering effects of a Rho kinase inhibitor, ripasudil (K-115), over 24 hours in primary open-angle glaucoma and ocular hypertension: a randomized, open-label, crossover study, Acta Ophthalmol. 93 (2015) e254–e260.
[8] S. Emi, I. Waka, S. Tamaki, et al., Evaluation of offset of conjunctival hyperemia induced by a Rho-kinase inhibitor; 0.4% Ripasudil ophthalmic solution clinical trial, Sci. Rep. 9 (2019) 3755, https://doi.org/10.1038/s41598–019–40255–9.
[9] T.M. Shaarawy, M.B. Sherwood, F. Grehn, Guidelines on Design and Reporting of Glaucoma Surgical Trials. Amsterdam, the Neth-Erlands, Kugler Publications, 2009, pp. 15–24.
[10] R.N. Weinreb, T. Aung, F.A. Medeiros, The pathophysiology and treatment of glaucoma: a review, JAMA 311 (2014) 1901–1911.

[11] G.L. Skuta, R.K. Parrish, Wound healing in glaucoma filtering surgery, Surv. Ophthalmol. 32 (1987) 149–170.
[12] D.C. Broadway, L.P. Chang, Trabeculectomy, risk factors for failure and the preoperative state of the conjunctiva, J. Glaucoma 10 (2001) 237–249.
[13] R.K. Shetty, L. Wartluft, M.R. Moster, Slit-lamp needle revision of failed filtering blebs using high-dose mitomycin C, J. Glaucoma 14 (2005) 52–56.
[14] H.A. Maestrini, S. Cronemberger, H.D. Matoso, et al., Late needling of flat filtering blebs with adjunctive mitomycin C: efficacy and safety for the corneal endothelium, Ophthalmology 118 (2011) 755–762.
[15] M.B. Sherwood, G.L. Spaeth, S.T. Simmons, et al., Cysts of Tenon's capsule following filtration surgery. Medical management, Arch. Ophthalmol. 105 (1987) 1517–1521.
[16] A.C.Y. Chew, H.M. Htoon, S.A. Perera, et al., Outcomes of surgical bleb revision at a tertiary Singapore eye hospital, Int. Ophthalmol. 42 (2022) 443–453.
[17] K. Mercieca, B. Drury, A. Bhargava, et al., Trabeculectomy bleb needling and antimetabolite administration practices in the UK: a glaucoma specialist national survey, Br. J. Ophthalmol. 102 (2018) 1244–1247.
[18] R.H. Ewing, R.L. Stamper, Needle revision with and without 5-fluorouracil for the treatment of failed filteringblebs, Am. J. Ophthalmol. 110 (1990) 254–259.
[19] P.G. Mardelli, C.M. Lederer, P.L. Murray, et al., Slit-lamp needle revision of failed filtering blebs using mitomycin C, Ophthalmol-ogy 103 (1996) 1946. –55.
[20] C. Xuhao, S. Lingge, H. Ying, et al., Safety and efficacy of bleb needling with antimetabolite after trabeculectomy failure in glau-coma patients: a systemic review and meta-analysis, J. Ophthalmol. 30 (2020), 4310258.
[21] M. Fagerli, K.T. Løfors, T. Elsås, Needling revision of failed filtering blebs after trabeculectomy: a retrospective study, Acta Ophthalmol. Scand. 81 (2003) 577–582.
[22] E.M. Ghoneim, M. Abd El Hameed, Needling augmented with topical application of mitomycin C for management of bleb failure, J. Glaucoma 20 (2011) 528–532.
[23] W. Liu, J. Wang, M. Zhang, et al., Comparison of subconjunctival mitomycin C and 5-fluorouracil injection for needle revision of early failed trabeculectomy blebs, J. Ophthalmol. 2016 (2016), 3762674.
[24] N. Anand, A. Khan, Long-term outcomes of needle revision of trabeculectomy blebs with mitomycin C and 5-fluorouracil: a comparative safety and efficacy report, J. Glaucoma 18 (2009) 513–520.
[25] N. Palejwala, P. Ichhpujani, G. Fakhraie, et al., Single needle revision of failing filtration blebs: a retrospective comparative case series with 5-fluorouracil and mitomycin C, Eur. J. Ophthalmol. 20 (2010) 1026–1034.
[26] J.S. Kim, H.J. Kim, K.I. Na, et al., Comparison of efficacy and safety of bleb needle revision with and without 5-fluorouracil for failing trabeculectomy bleb, J. Glaucoma 28 (2019) 386–391.
[27] A. Halili, L. Kessel, Y. Subhi, et al., Needling after trabeculectomy—does augmentation by anti-metabolites provide better out-comes and is mitomycin C better than 5-Fluoruracil? A systematic review with network meta-analyses, Acta Ophthalmol. 98 (2020) 643–653.
[28] L. Chang, J.G. Crowston, M.F. Cordeiro, et al., The role of the immune system in conjunctival wound healing after glaucoma sur-gery, Surv. Ophthalmol. 45 (2000) 49–68.
[29] A. Akhmetshina, C. Dees, M. Pileckyte, et al., Rho-associated kinases are crucial for myofibroblast differentiation and pro-duction of extracellular matrix in scleroderma fibroblasts, Arthritis Rheum. 58 (2008) 2553–2564.