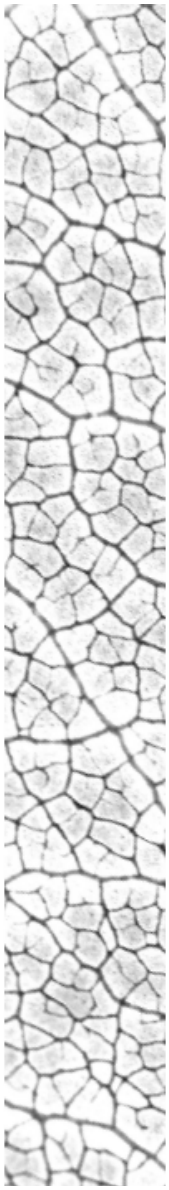

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Rho-kinase inhibitor Y-27632 on Schlemm's canal diameter in a juvenile rat model injected with sodium hyaluronate

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Abstract

Background: The purpose of this study was to examine the effects of the rho-kinase inhibitor Y-27632 on the diameter of the Schlemm's canal in a juvenile rat model injected with sodium hyaluronate.

Material and Methods: This was an original experimental study with a post-test control group design. Eyeballs of rats aged 4-6 months were used in this study. Samples were divided into six groups: negative control, positive control I with intracameral sodium hyaluronate injection, positive control II with topical Y-27632 10 mM, and three experimental groups with intracameral injections of sodium hyaluronate and Y-27632 10^{-1} mM, 1 mM, and 10 mM, respectively. Changes of the Schlemm's canal diameter were evaluated. Quantitative measurements were taken using computerized image analysis with the dot slide program.

Results: There were statistically significant differences among the control and experimental groups ($p < 0.05$). The greatest increase in diameter of the Schlemm's canal was observed in the experimental group given sodium hyaluronate and Y-27632 10 mM, with a mean value of 118.42 µm.

Conclusion: The rho-kinase inhibitor Y-27632 increased the diameter of the Schlemm's canal in juvenile rats injected with sodium hyaluronate.

Keywords: Juvenile rat model, rho-kinase inhibitor (Y-27632), Schlemm's canal, sodium hyaluronate

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BACKGROUND

The trabecular meshwork and Schlemm's canal contribute to maintain the outflow of aqueous humor. Each portion of the trabecular meshwork consists of cells and extracellular matrix. The cells are enriched with smooth muscle-like components with electromechanical functions and express several specific proteins. The trabecular meshwork is derived from the neural crest in the fourth month of gestation. The complete morphogenesis of the trabecular meshwork is achieved around birth, while maturation will be completed during the first eight years of life (Gasiorowzki and Russell 2009, Tamm 2009, Yu et al. 2011).

There are three important structures of the trabecular meshwork related to aqueous humor outflow regulation, the extracellular matrix in the juxtacanalicular region, the inner wall of the Schlemm's canal, and its lumen (Acot and Kelley 2008, Rhee et al. 2009).

Rho-kinase protein is a protein that maintains the physiologic function of the trabecular meshwork. It contributes to the regulation of actomyosin organization and extracellular matrix synthesis. The modulation of these systems alters the contractility and adhesion of the trabecular meshwork cells (Tian et al. 2009).

Several specific rho-kinase inhibitors have been shown to induce inhibition of actomyosin activity and modulate the trabecular meshwork and Schlemm's canal morphology. Y-27632 is noted as one of these protein inhibitors. Honjo et al. concluded that Y-27632 significantly reduced the intraocular pressure in rabbits. Cellular changes to the trabecular meshwork and ciliary muscle relaxation were considered to be the underlying mechanisms (Honjo et al. 2001, Ishizaki et al. 2000).

Y-27632 has been used widely in experimental research in adults, both in vivo and in vitro. Benozzi stated that sodium hyaluronate injections increased the density of the extracellular matrix and decreased the diameter of the flow channels (Benozzi et al. 2002).

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the Schlemm's canal in a juvenile rat model with sodium hyaluronate injection.

MATERIALS AND METHODS

Animals

This study was conducted in the Physiology Laboratory of the Medical Faculty of Brawijaya University, Malang, from July 2012 to October 2012. Twenty-four juvenile Wistar rats aged 4-6 weeks were included in this study.

Preparation of the Eyes

The sample was divided into six groups. The first group was the negative control group without any treatment. The second to fifth groups were anesthetized with ketamine hydrochloride (50 mg/kg) (Hameln pharmaceuticals, Germany) administered intraperitoneally. Next, 15 µl of sodium hyaluronate (BBTVisc 1.5%, Bohus BioTech) was injected into each eye of the anesthetized rats with a syringe and a 30-gauge needle. The eyes were then focused under a surgical microscope (DECA-21, Inami & Co. Ltd., Model-0940SD, Made in Japan) with coaxial light. The needle was moved through the corneoscleral limbus to the anterior chamber with the bevel down. When the tip of the bevel reached the anterior chamber, the liquid progressively increased the chamber's depth, separating the needle from the iris and avoiding needle–lens contact. Applications were made slowly but using a force sufficient to just empty the syringe content. Antibiotic eye ointment was applied after the injection.

Application of one drop of 0.5% proparacaine hydrochloride was performed 24 hours later on each eye. The second group received no other treatment. The third to fifth group were given topical rho-kinase inhibitor (Y-27632) at a concentration of 0.1 mM, 1 mM, and 10 mM, respectively. Each treatment was administered to the central cornea as four 3 ml drops, at intervals of 30 seconds, with blinking prevented between and after the last drops. The last group was only treated with Y-27632 10 mM topically. All animals were sacrificed three hours after treatment.

Eyes were enucleated immediately after the rats were sacrificed, then subdivided at the equator into anterior and posterior segment. The eyes were fixed in b4% paraformaldehyde (PFA). The anterior segments were subdivided into quadrants after careful removal of the lens. The specimens including Schlemm's canal were prepared and embedded in paraffin.

Histological Analysis

Photographs of the microscopic sections were taken and analyzed using the automated image and dot slide program, at magnification of 400x. In the meridional sections, measurements were taken of the anterior–posterior diameter of the Schlemm's canal. Measurements were taken four times and the average value is given in µm.

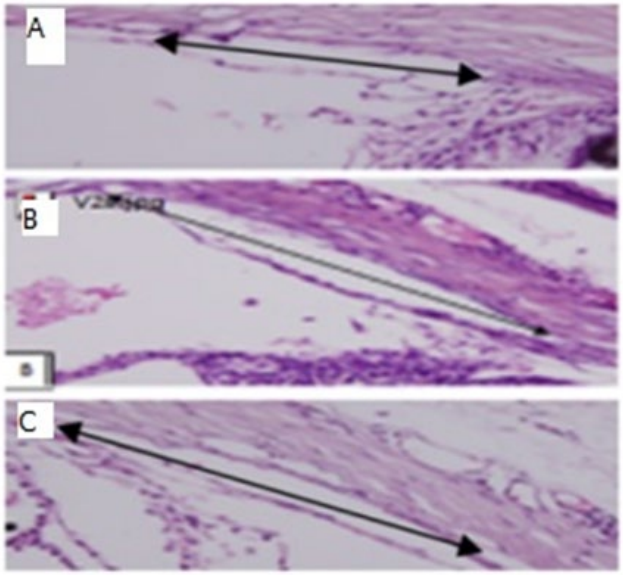

Fig. 1. Schlemm's canal diameter in treatment groups (400x). A. Schlemm's canal in Y-27632 10-1 mM group; B. Schlemm's canal in Y-27632 1 mM group; C. Schlemm's canal in Y-27632 10 mM group

Table 1. The average of Schlemm's canal diameter in all groups

Group	Mean ± SD (µm)
Control (-)	76.7500 ± 2.5371
Control (+) I	63.4500 ± 1.1091
Control (+) II	83.0250 ± 4.3007
10^{-1} mM Y-27632	98.6500 ± 1.9975
1 mM Y-27632	108.2802 ± 5.0599
10 mM Y-27632	118.7202 ± 1.7914

Statistics

Data were analyzed using independent t-tests, one-way ANOVA, and multiple comparison tests with LSD. P-value < 0.05 was considered to be statistically significant.

RESULTS

Histologically, the Schlemm's canal appeared as an ellipse space covered with an endothelium layer colored in pink and purple nucleus. Increased diameter of the Schlemm's canal lumen was noted in groups administered Y-27632 (**Fig. 1**). Increasing Schlemm's canal diameter was seen with increasing concentrations of Y-27632, as shown in **Table 1**.

Independent samples t-tests revealed significant differences between the negative control group and the positive I control group receiving only sodium hyaluronate injection. The differences were seen in all variables measured ($p < 0.05$).

The same results were also seen when comparing the negative control group and the positive II control group receiving only Y-27632 10 mM topically. The differences were also significant in all variables measured ($p < 0.05$), as shown in **Table 2** and **Table 3**.

Table 2. Results of one-way ANOVA between positive I control group and treatment groups

Variables	Groups	Mean ± SD (µm)	p-value
Schlemm's canal diameter	Control (+) I	63.4500 ± 1.1091	
	10^{-1} mM Y-27632	98.6500 ± 1.9975	0.000
	1 mM Y-27632	108.2802 ± 5.0599	
	10 mM Y-27632	118.7202 ± 1.7914	

Table 3. Results of one-way ANOVA between positive II control group and treatment groups

Variables	Groups	Mean ± SD (µm)	*p-value*
Schlemm's canal diameter	Control (+) II	83.0250 ± 4.3007	
	10^{-1} mM Y-27632	98.6500 ± 1.9975	0.000
	1 mM Y-27632	108.2802 ± 5.0599	
	10 mM Y-27632	118.7202 ± 1.7914	

One-way ANOVA showed significant differences in the diameter of the Schlemm's canal between the positive I control group and the treatment groups ($p < 0.05$) (**Table 2**). One-way ANOVA between the positive II control group and the treatment groups also showed significant differences in all variables measured ($p < 0.005$) (**Table 3**).

DISCUSSION

Honjo et al. conducted a similar study with Y-27632. The chosen concentrations were 10 and 100 mM so as to provide the desired concentration, with the assumption that only 1% of the drug would penetrate intracameral in approximately 125 µl of rabbit anterior chamber volume. The volume of the anterior chamber in rats in this study was 15 µl; therefore, we used 1/10 concentration of the previous study (Honjo et al. 2001).

The increasing intraocular pressure effect caused by injection of sodium hyaluronate was noted after 24 hours and lasted for five days in the study conducted by Benozzi. We also used the same 24 hours after injection to perform topical application of Y-27632. Honjo et al. also reported that Y-27632 decreased intraocular pressure after 30 minutes and reached a peak 1-3 hours after application. In the current study, the evaluation was conducted three hours after Y-27632 administration in order to gain optimal effect (Benozzi et al. 2002, Honjo et al. 2001).

The largest average of Schlemm's canal diameter was observed in the treatment group receiving Y-27632 10 mM. Kameda reported that Y-27632 applied in monkey eyes increased the permeability of the endothelial layer of the Schlemm's canal, linked to the disruption of tight junctions and actin depolymerization. Cellular processes are thought to be the mechanism of change in the diameter of the Schlemm's canal (Kameda et al. 2012).

Rao et al. reported that Y-27632 increased permeability and produced changes in cell adhesion and relaxation of the trabecular meshwork. Several mechanisms are thought to underlie the morphological changes as observed in this study (Rao et al. 2001).

Shen et al. reported that the regulation of protein rho-kinase is essential for cytoskeletal modulation of the trabecular meshwork. Thus, administration of a rho-kinase inhibitor, in this case is Y-27632, will inhibit the activities generated by the function of the protein itself, and will change the morphology of trabecular meshwork cells (Shen et al. 2008).

Y-27632 at a single concentration of 10 causes morphological changes in the trabecular meshwork. Temming reported that protein kinase inhibitors may have a low level of cellular selectivity. Y-27632 is also able to affect normal cells as well. Unfortunately, there is no expression of the target receptor in normal cells; therefore, normal cells cannot conjugate with the drug and its carrier. This condition leads to no accumulation, which in turn minimizes the effects of the drug (Temming et al. 2008).

There were significant differences among the groups treated with various concentrations of Y-27632, as well as among the treated groups and the control groups. This indicated that the range of concentrations of Y-27632 produced enough morphological changes to observe significant differences. The most substantial changes were observed in the group receiving Y-27632 at the highest concentration of 10 mM.

In this study, measurements were performed using a computerized dot-slide program; one observer performed the measurements and repeated them four times. The average of these measurements was then taken. Instrumentation with reliable and objective measurement provides good internal validity. Randomization was also performed for sample selection. There were no rats that died or were excluded from this study. Mortality or drop out in the sample would reduce the internal validity of the study. The use of control groups in experimental research supports the external validity. There were two positive control groups and one negative control group used in this study. Given all of the reasoning above, it can be assumed that the validity of this research is quite good (Festing and Altman 2002).

We may conclude from this study that there is a tendency for greater changes in the diameter of Schlemm's canal with increasing concentrations of Y-27632. However, we are not able to determine the optimal concentration of Y-27632 to provide effective diameter changes of the Schlemm's canal. It may be caused by the linear changes of Schlemm's canal with the increasing concentrations given. Further research is still needed with higher and more varied concentrations to evaluate the optimal concentration of Y-27632 to produce morphological changes in the Schlemm's canal.

The exact mechanism of morphological changes in the Schlemm's canal due to administration of Y-27632 was not directly evaluated in this study as the molecular examination was also not performed. However, several related studies may explain the underlying mechanisms.

This study provides the basic research for further evaluation of molecular changes in the Schlemm's canal due to Y-27632 administration.

CONCLUSION

It can be concluded that the administration of the rho-kinase inhibitor Y-27632 in a juvenile rat model injected with sodium hyaluronate caused an increased diameter of the Schlemm's canal. We hoped this agent might be an alternative medical therapy for lowering intraocular pressure related to facilitation of aqueous humor through modulation of the trabecular meshwork. Further research is still needed to evaluate the effectiveness and molecular changes of the rho-kinase inhibitor Y-27632 in modulation of the Schlemm's canal.

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REFERENCES

Acot TS, Kelley MJ (2008) Extracellular Matrix in the Trabecular Meshwork. Exp Eye Res, 86(4): 543–561. https://doi.org/10.1016/j.exer.2008.01.013

Benozzi J, Nahum LP, Campanelli JL, Rosenstein RE (2002) Effect of Hyaluronic Acid on Intraocular Pressure in Rats. Invest Ophthalmol Vis Sci, 43(7): 2196-2200.

Festing MF, Altman DG (2002) Guidelines for the Design and Statistical Analysis of Experiments Using Laboratory Animals. ILAR J, 43(4): 244-258. https://doi.org/10.1093/ilar.43.4.244

Gasiorowzki JZ, Russell P (2009) Biological properties of trabecular meshwork cells. Exp Eye Res, 88(4): 671–675. https://doi.org/10.1016/j.exer.2008.08.006

Honjo M, Tanihara H, Inatani M, Kido N, Sawamura T, Yue BY, Narumiya S, Honda Y (2001) Effects of Rho-Associated Protein Kinase Inhibitor Y-27632 on Intraocular Pressure and Outflow Facility. Invest Ophthalmol Vis. Sci, 42(1): 137-144.

Ishizaki T, Uehata M, Tamechika I, Keel J, Nonomura K, Maekawa M, Narumiya S (2000) Pharmacological Properties of Y-27632, a Specific Inhibitor of Rho-Associated Kinases. Mol Pharmacol, 57(5): 976–983.

Kameda T, Inoue T, Inatani M, Fujimoto T, Honjo M, Kasaoka N, Inoue-Mochita M, Yoshimura N, Tanihara H (2012) The Effect of Rho-associated Protein Kinase Inhibitor on Monkey Schlemm's Canal Endothelial Cells. Invest Ophthalmol Vis Sci, 53(6): 3092-3103. https://doi.org/10.1167/iovs.11-8018

Rao PV, Deng PF, Kumar J, Epstein DL (2001) Modulation of Aqueous Humor Outflow Facility by the Rho-Kinase Specific Inhibitor Y-27632. Invest Ophthalmol Vis Sci, 42(5): 1029-1037.

Rhee DJ, Haddadin RI, Kang MH, Oh DJ (2009) Matricellular proteins in the trabecular meshwork. Exp Eye Res, 88(4): 694–703. https://doi.org/10.1016/j.exer.2008.11.032

Shen X, Koga T, Park BC, SundarRaj N, Yue BY (2008) Rho GTPase and cAMP/Protein Kinase A Signaling Mediates Myocillin-induced Alterations in Cultured Human Trabecular Meshwork Cells. J Biol Chem, 283(1): 603-612. https://doi.org/10.1074/jbc.M708250200

Tamm ER (2009) The trabecular meshwork outflow pathways: Structural and functional aspects. Exp Eye Res, 88(4): 648–655. https://doi.org/10.1016/j.exer.2009.02.007

Temming K, Fretz MM, Kok RJ (2008) Organ and Cell-Type Specific Deliver of Kinase Inhibitors: A Novel Approach in the Development of Targeted Drugs. Curr Mol Pharmacol, 1(1): 1-12. https://doi.org/10.2174/1874-470210801010001

Tian B, Gabelt BT, Geiger B, Kaufman PL (2009) The Role of the Actomyosin System in Regulating Trabecular Fluid Outflow. Exp Eye Res, 88(4): 713–717. https://doi.org/10.1016/j.exer.2008.08.008

Yu WY, Sheridan C, Grierson I, Mason S, Kearns V, Lo ACY, Wong D (2011) Progenitors for the Corneal Endothelium and Trabecular Meshwork: A Potential Source for Personalized Stem Cell Therapyin Corneal Endothelial Diseases and Glaucoma. J Biomed Biotechnol: 1-13. https://doi.org/10.1155/2011/412743