Effectiveness of Retrobulbar Hyaluronidase Injection in an Iatrogenic Blindness Rabbit Model Using Hyaluronic Acid Filler Injection

Won Lee, M.D., Ph.D.
Wook Oh, M.D.
Hyung-Seok Ko, M.D.
Sang-Young Lee, M.D.
Ki Wook Kim, M.D.
Eun-Jung Yang, M.D., Ph.D.

Anyang, Seoul, and Cheonju, Republic of Korea

Background: Blindness caused by soft-tissue filler injection is the most tragic complication, with no standard treatments until recently. Retrobulbar hyaluronidase injection has been proposed as the treatment, but its effectiveness in visual compromise remains to be determined. The authors aimed to determine the effectiveness of retrobulbar hyaluronidase using soft-tissue filler in an iatrogenic blindness animal model.

Methods: New Zealand White rabbits were used to simulate the hyaluronic acid–associated vascular occlusion model. A volume of 0.7 to 1.6 ml of hyaluronic acid filler was injected into the internal carotid artery to create a retinal artery occlusion. The rabbits were administered retrobulbar hyaluronidase (3000 IU) at different postobstruction time points (5 and 10 minutes). No intervention was given to the control group. Fundus photography was performed before and immediately after the filler injection and immediately after the administration of retrobulbar hyaluronidase. Electroretinography was performed after 60 minutes to confirm the retinal reperfusion and electrophysiologic function.

Results: All of the experimental eyes recorded total occlusion after hyaluronic acid injection. Three eyes with a completely occluded retinal artery following retrobulbar hyaluronidase treatment showed improved retinal reperfusion by fundus photography and corresponding electroretinography. Despite administration of the retrobulbar hyaluronidase injection, one completely occluded eye showed no improvement in perfusion. All of the control eyes recorded complete occlusion 1 hour after hyaluronic acid filler injection.

Conclusions: Retrobulbar hyaluronidase may be an effective evidence-based treatment option for humans. Hyaluronidase concentration and injection time are the important factors for faster recovery, but additional studies are still required. (*Plast. Reconstr. Surg.* 144: 137, 2019.)

Soft-tissue filler injection is the second most commonly performed cosmetic procedure following botulinum toxin treatment. As the field of soft-tissue augmentation has become increasingly popular, reports of adverse events have increased. Visual compromise—including loss of vision, ophthalmoplegia, and ptosis—is not common, but complications caused by hyaluronic acid filler injection have been constantly reported.[1] Unfortunately, even with early recognition, the visual loss associated with filler injections is mostly irreversible, and no proven treatments are available yet.

Hyaluronidase is obtained from animal, leech, or bacterial enzymes. It is extracted from ovine or bovine testicles or from recombinant DNA and is used for better drug dispersion by destroying human hyaluronic acid. However, it is also used for resolving the unpleasant results of hyaluronic acid filler nowadays. Hyaluronidase has been shown to dissolve the hyaluronic acid filler present in the blood vessel as it spreads subcutaneously.[2,3]

From Yonsei E1 Plastic Surgery Clinic; Samsung Feel Clinic; Samsung Lohas Clinic; Korea Leaders Medical Group; Regen Clinic; and the Department of Plastic and Reconstructive Surgery, Hanyang University Seoul Hospital, Hanyang University, College of Medicine.

Received for publication August 14, 2018; accepted December 11, 2018.

DOI: 10.1097/PRS.0000000000005716

Disclosure: *Dr. Won Lee has been an investigator, speaker, and consultant for JETEMA Co., Ltd., South Korea. The hyaluronic acid fillers, e.p.t.q. S100, were sponsored by JETEMA. The remaining authors have no financial interest to declare in relation to the content of this article.*

Retrobulbar hyaluronidase has been the proposed treatment in various articles but, until now, has not been scientifically proven.[2,4] A report demonstrated that vision has been restored using this method in a patient suspected of hyaluronic acid filler–induced blindness.[5] Although subcutaneously injecting hyaluronidase is known to be effective, whether retrobulbar hyaluronidase can actually reach the ophthalmic artery branch remains to be elucidated. The authors injected hyaluronic acid in the rabbit eye to introduce blindness and then confirm whether it can be recovered by retrobulbar hyaluronidase administration. If retrobulbar hyaluronidase is proven effective, even though further study is required before its clinical application, it could be considered as an evidence-based therapeutic option. Therefore, this study aimed to determine the effects of retrobulbar hyaluronidase on hyaluronic acid–induced retinal occlusion in a rabbit model.

MATERIALS AND METHODS

Retinal Artery Occlusion Model

Animal experiments were performed according to the Good Laboratory Practice guidelines in the Centralbio Research Institute, Republic of Korea. New Zealand White rabbits (Samtako Bio Korea, Osan, Republic of Korea), weighing 2.3 to 2.6 kg, were selected because their ocular vascular anatomy is similar to that of humans.[6] The rabbits were placed under intravenous anesthesia and monitored throughout the study period. Anesthesia was induced by intravenously injecting Zoletil 50 (Virbac, Carros, France) and xylazine (Rompun; Bayer AG, Leverkusen, Germany). Zoletil and xylazine are mixed 1:1 and injected 0.1 ml/kg intravenously. When extending the anesthesia, an additional of one-half to one-third of the initial anesthetic dose was administered. Fundus photography (TRC-501X Retinal Camera; Topcon Corp., Tokyo, Japan) was performed to confirm the retinal perfusion in all eyes at baseline.

A midline neck incision was made. The thyroid cartilage was exposed, and dissection was performed laterally to locate the internal carotid artery. All left eyes of the rabbits were tested because of the possibility of animal death during the experiment when occlusion was performed at the bilateral internal carotid artery. Cannulation was performed in the internal carotid artery and ligated for further filler injection. After cannulation, C-arm fluoroscopy (Arcdis Varix; Siemens Co., Berlin, Germany) for proper arterial flow to the radial artery was performed. Radiologic findings showed that the contrast agent reached the ophthalmic artery (Fig. 1). Then, 0.5 ml of hyaluronic acid filler (e.p.t.q. S100; JETEMA Co., Ltd., Seoul, Republic of Korea) was placed in a 1-ml syringe with a 30-gauge needle. The hyaluronic acid was injected into the radial artery through the ophthalmic artery. Only the right ophthalmic artery/radial artery was obstructed, and the left side was left uncompromised. Retinal ischemia was confirmed by fundus photography in real time. When fundus images showed vascular occlusion changes, we stopped filler injection; otherwise, we repeated filler injection slowly to establish vascular occlusion up to 1.6 ml. Fundus measurements were then repeated immediately after occlusion of the radial artery.

Retrobulbar Hyaluronidase Injection

After confirming the occlusion, the authors injected retrobulbar hyaluronidase into the rabbits that had undergone radial artery occlusions. In our preliminary study, we tested 1500 IU of hyaluronidase mixed with 3 ml of normal saline but could not see improvement at the fundus; therefore, we tried 3000 IU of high-dose hyaluronidase mixed with 2 ml of normal saline and could see reperfusion on funduscopic images. Based on the preliminary test results, 2 ml of 3000 IU hyaluronidase (Liporase; Daehan New Pharm Co., Hwaseong, Republic of Korea) was injected into the retrobulbar area using a 26-gauge, long needle 5 minutes after hyaluronic acid injection in two rabbits and 10 minutes after injection in the other

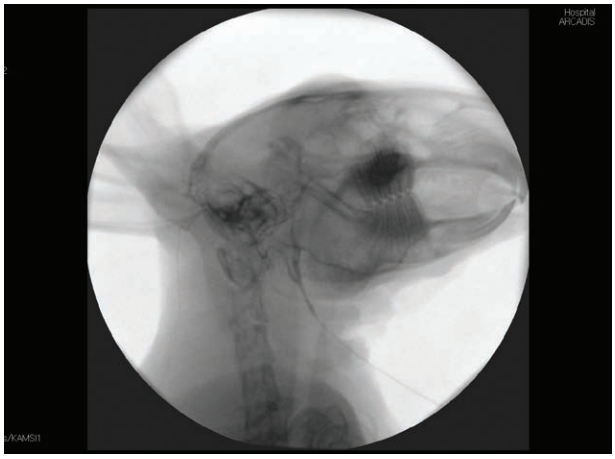

Fig. 1. Radiologic imaging showed that the contrast agent reached the ophthalmic artery. Cannulation was performed in the internal carotid artery and ligated for further filler injection. After cannulation, C-arm fluoroscopy for proper arterial flow to the ophthalmic artery was performed.

two rabbits. Radial artery perfusion in the experimental groups determined from the fundus photographs immediately after the administration of retrobulbar hyaluronidase and the corresponding full-field flash electroretinographic (HMsERG model 200; OcuSciences, Inc., Ann Arbor, Mich.) readings were documented 60 minutes after the administration of retrobulbar hyaluronidase.

Control groups were operated on for wound closure after the hyaluronic acid injection and were monitored with electroretinography after 60 minutes for the possibility of natural healing. All rabbits were killed with 0.1 ml/kg of succinylcholine administered intravenously.

RESULTS

Radiologic findings showed that the contrast agent reached the ophthalmic artery in all eyes (Fig. 1). A total of six eyes were included in this study: two control and four experimental. All eyes with occluded radial artery demonstrated the absence of retinal perfusion on fundus photography. Among the four eyes with a completely occluded radial artery, three showed improvement in retinal perfusion, with normal electroretinography showing an a-wave form (initial negative deflection) followed by a b-wave form (positive deflection) at 60 minutes after retrobulbar hyaluronidase injection (3000 IU); the magnitude of response depends on the subject. One animal showed no improvement in the perfusion by fundus photography and the corresponding abnormal electroretinographic readings (Figs. 2 and 3). A summary of the findings is shown in Table 1.

Two control eyes had hyaluronic acid filler–induced radial artery occlusion, which showed no

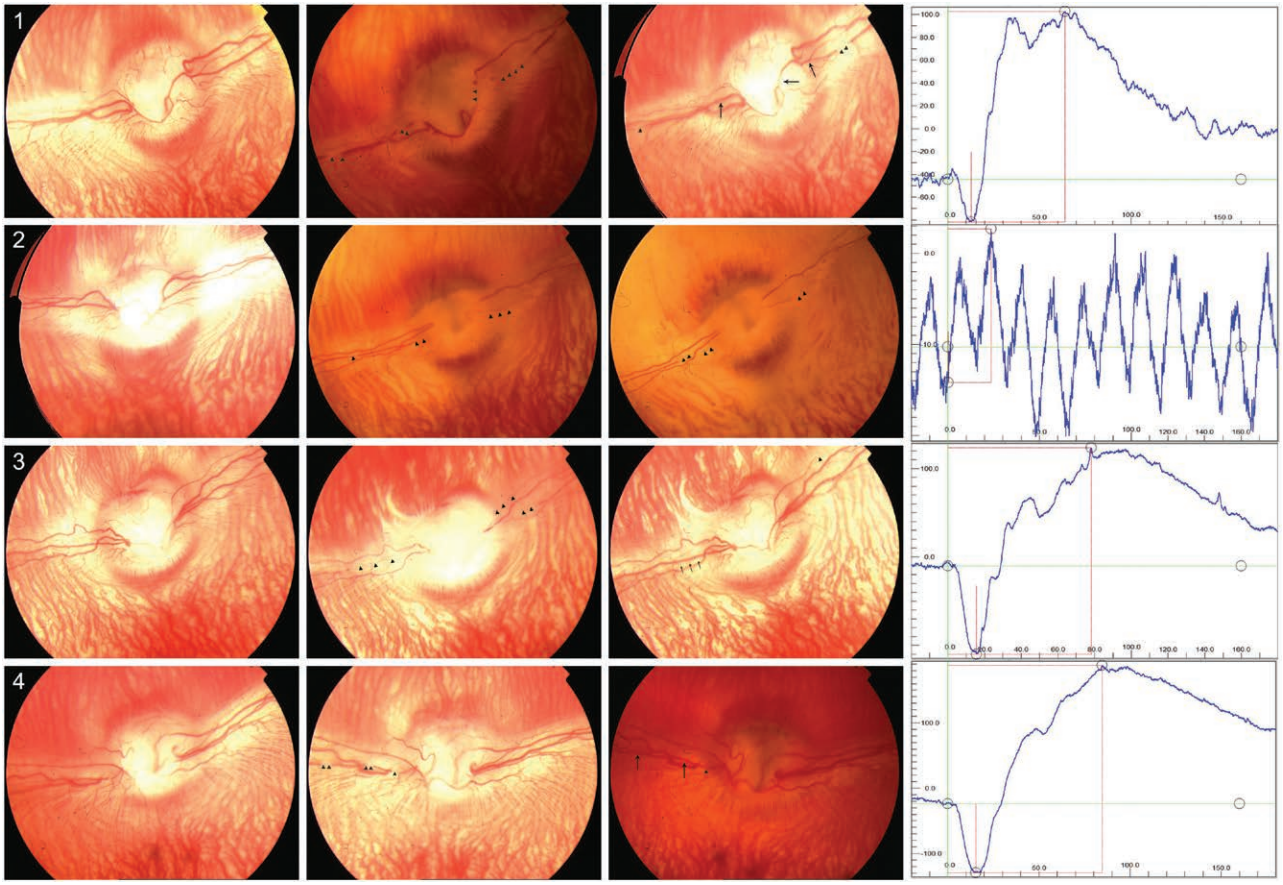

Fig. 2. Fundus photographs before and after hyaluronic acid injection, and after injection of 3000 IU of retrobulbar hyaluronidase in the experimental group from left to right (*left*). Before intraarterial injection, all fundi appeared intact and can be seen clearly. Retinal arterial occlusion (*arrowhead*) was on funduscopic images after hyaluronic acid injection. Fundus reperfusion (*arrow*) image after retrobulbar hyaluronidase of three experimental cases (cases 1, 3, and 4) and fundus retinal occlusion image after retrobulbar hyaluronidase in one experimental case (case 2) are shown. Corresponding normal electroretinographic finding after retrobulbar hyaluronidase of three experimental cases and abnormal electroretinogram after retrobulbar hyaluronidase injection in one experimental case (*right*) are shown.

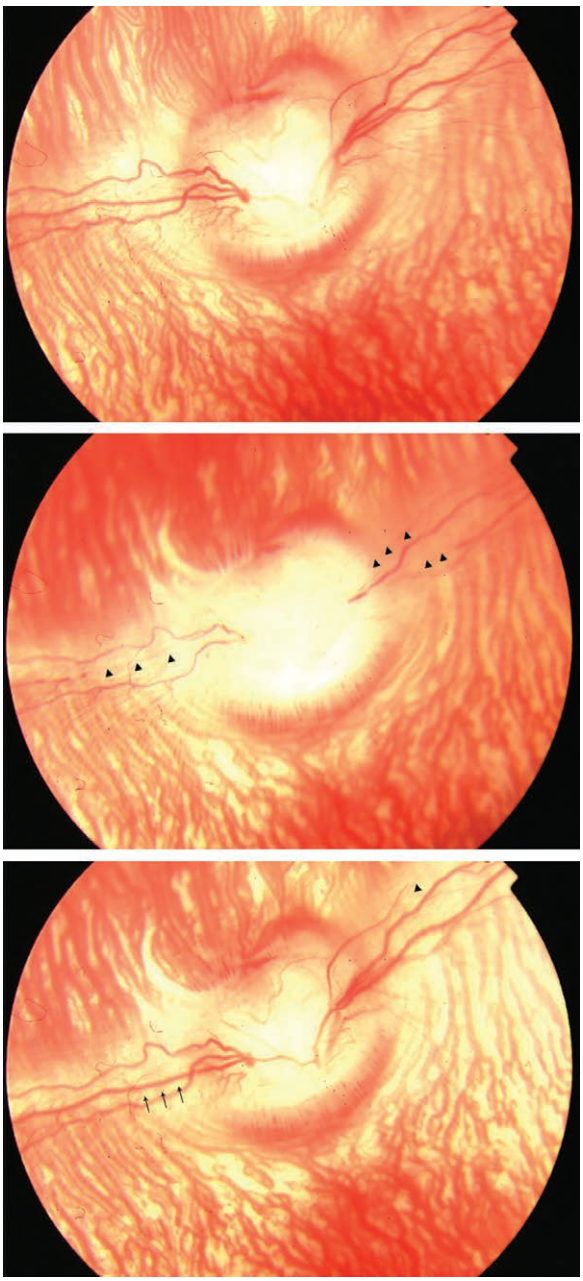

Fig. 3. Fundus photographs of the eye of case 3 before (*above*), after injection of 0.9 ml of hyaluronic acid (*center*), and after injection of 3000 IU of retrobulbar hyaluronidase (*below*). Before intraarterial injection, all fundi appeared intact and can be clearly seen. Prominent retinal arterial occlusion (*arrowhead*) was found. A relatively intact retinal vein was found. Funduscopic images obtained 10 minutes after retrobulbar hyaluronidase injection show reperfusion (*arrows*).

changes in the retinal occlusion on electroretinography 60 minutes after hyaluronic acid filler injection (Fig. 4). There was almost no response to light in the control group. The a-wave response was significantly lower in the control group than in the experimental group, and there was almost no b-wave response (Fig. 5).

DISCUSSION

There is no proven effective treatment for blindness following hyaluronic acid injection. Retrobulbar hyaluronidase injection has been proposed as the treatment, but its effectiveness remains to be determined. This study aimed to investigate the effectiveness of retrobulbar hyaluronidase in a rabbit retinal obstruction model.

Experimental Obstruction Model

The vasculature of the rabbit eye is known to be similar to that of humans.[6] However, unlike in humans, animals have more potential healing capability; thus, creating an iatrogenic vascular occlusion is difficult. In our preliminary study, we attempted to create an obstruction model by mixing filler and normal saline at a ratio of 1:1 to reduce viscosity, as in the case of a previous article.[7] However, we failed to induce obstruction, and we observed that some of the occluded vessels were spontaneously healed without injecting hyaluronidase. Through a series of experiments, the authors found that the concentration and viscosity of the filler were important for inducing obstruction. A previous study reported the efficacy of injecting intravenous hyaluronidase after induction of an obstructive model after injecting approximately 0.35 ml of filler without dilution in the whole experimental group.[6] Subjects in the control group were given saline, and two of 10 cases showed partial reperfusion. We suspect that some of the restored cases in the experimental group of that study are attributable not to the treatment but to spontaneous reperfusion. In our preliminary experiment, the rabbit was administered 0.3 ml of hyaluronic acid (e.p.t.q. S50) for retinal occlusion. However, the artery recovered naturally; thus, 0.3 ml of hyaluronic acid (e.p.t.q. S100) mixed with normal saline was administered. The artery still recovered naturally, and the occlusion model was not successfully created. Finally, we injected 0.3 ml of hyaluronic acid (e.p.t.q. S100) without dilution and the vascular occlusion was created. In our current preclinical study, the authors have induced obstruction by infusing 0.5 ml of hyaluronic acid filler without dilution and then repeated a 0.2- to 0.3-ml filler injection to establish vascular obstruction. Blindness in rabbits could have been induced only when a sufficient amount of hyaluronic acid (0.7 to 1.6 ml) was injected, and we have confirmed

Table 1. Summary of the Experiment

	Weight (kg)	HA Filler (ml)	Time (min) to Hyaluronidase Injection	Hyaluronidase Concentration (IU)	ERG Waveform Response*	a-Wave (μV)*	b-Wave (μV)*
Control	2.6	0.9	—	Not injected	Abnormal	1.3	2.3
Control	2.62	0.7	—	Not injected	Abnormal	27	68.5
1	2.36	0.8	5	3000	Normal	47.8	168.5
2	2.5	1.4	5	3000	Abnormal	2.8	11.5
3	2.38	0.9	10	3000	Normal	102.8	254.5
4	2.6	1.6	10	3000	Normal	111.5	274

HA, hyaluronic acid; ERG, electroretinographic.
*Electroretinographic readings were documented 60 min after the administration of retrobulbar hyaluronidase in the experimental group. Animals in the control group were monitored with electroretinography after 60 min for the possibility of natural healing.

vascular obstruction from the fundus photographs in all subjects. We have proved persistent vascular obstruction by means of electroretinography in the subjects in the control group.

Rationale for Recanalization

The rationale behind retrobulbar hyaluronidase treatment is to reverse the ischemic course of the ophthalmic artery, retinal artery, posterior ciliary artery, and the arteries of the optic nerve through the retrobulbar space.[2,8] Because it has been demonstrated that hyaluronidase can diffuse into the obstructed vessels and degrade the hyaluronic acid filler,[3,9] retrobulbar hyaluronidase is expected to cross the vessel walls of these arteries (Fig. 6), and possible treatment recommendations have been described.[10] Hyaluronidase within the vessels would allow the enzyme to hydrolyze the hyaluronic acid and restore the perfusion of ischemic retina. However, whether hyaluronidase can reach the human retina and choroid remains unclear.

In our current study, fundus photography showed reperfusion of the radial artery and electroretinography showed normal response in three experimental cases. Administration of retrobulbar hyaluronidase to the ophthalmic artery may degrade the hyaluronic acid filler and allow the reestablishment of vascular perfusion. Because perfusion can improve without restoration of the retina, the experiment should have checked the functional test, such as electroretinography. Sufficiently bright flashes will elicit electroretinograms containing an a-wave form (initial negative deflection) followed by a b-wave (positive deflection). The magnitude of the response in three experimental cases was different but showed a normal

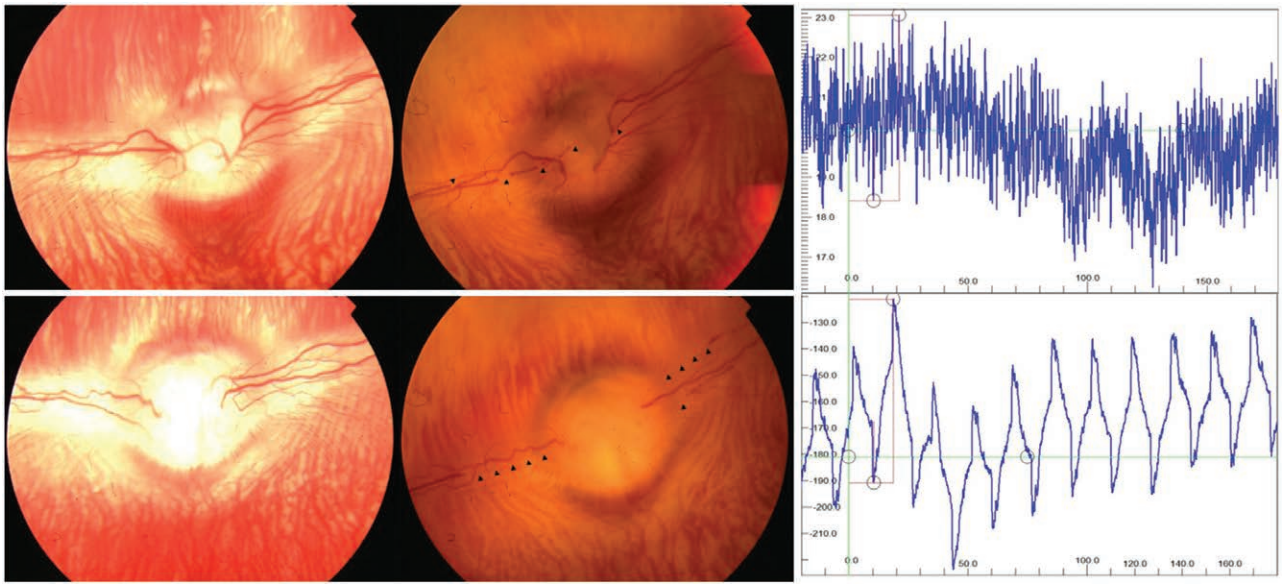

Fig. 4. Funduscopic images and electroretinographic findings in the control group. Before intraarterial injection, all fundus structures appeared intact and can be clearly seen (*left*). Retinal arterial occlusion (*arrowhead*) was found, and the fundus vasculature remained obstructed after intraarterial hyaluronic acid injection (*center*). Electroretinographic findings were abnormal 60 minutes after hyaluronic acid filler injection (*right*).

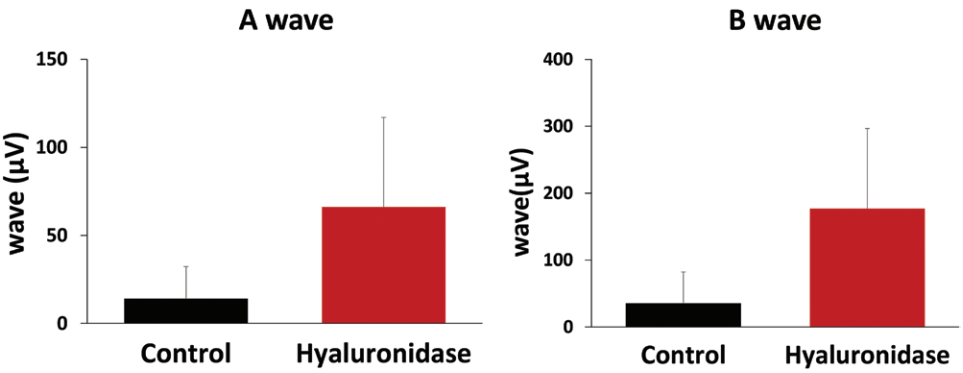

Fig. 5. Results of electroretinography. There was almost no response to light in the control group. The a-wave response was significantly lower in the control group than in the experimental group, and there was almost no b-wave response.

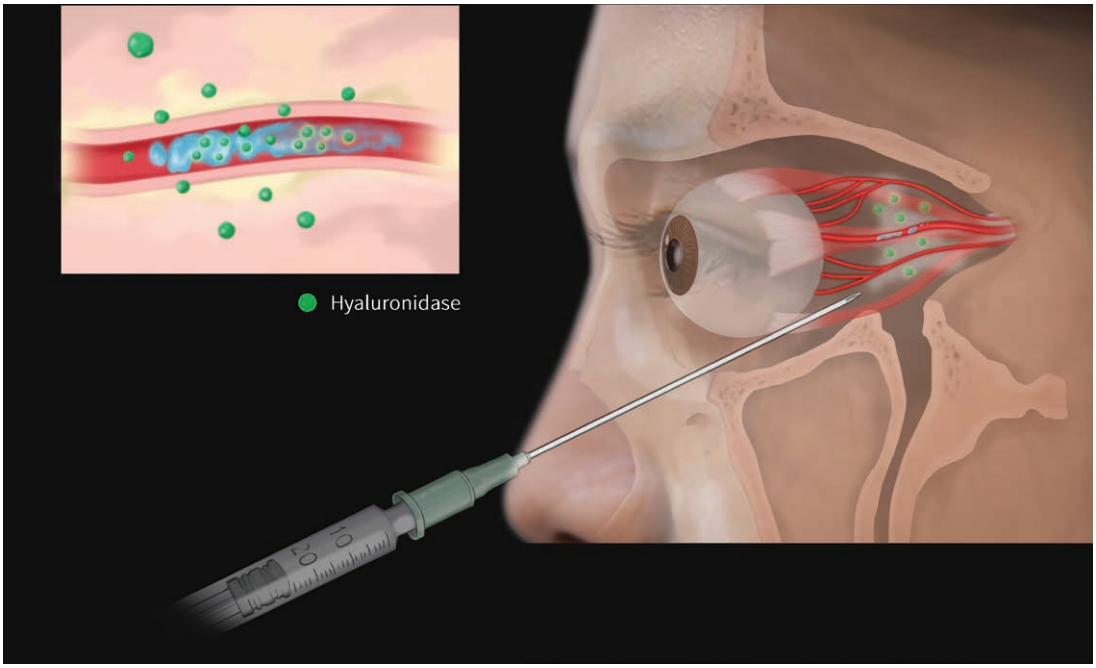

Fig. 6. Hyaluronidase diffuses through the arterial wall at some concentration, depending on the rate. To accomplish complete dissolution of filler obstruction (*blue*), the concentration of hyaluronidase in the right location should be sufficient for a long time to result in complete hydrolysis.

waveform (Fig. 2). The reason for failed reperfusion in one experimental case is presumably because the injected hyaluronic acid is too much to allow the hyaluronidase to degrade or the hyaluronidase did not work effectively. The ineffective effect of hyaluronidase may be attributable to its insufficient amount to completely degrade hyaluronic acid, or possibly it could not permeate sufficiently into the ophthalmic artery.

Timing of Hyaluronidase Administration

Recent literature insists that hyaluronidase would not be effective and showed four cases as evidence.[4] However, hyaluronidase was injected late, approximately 4 hours after blindness occurred. In our experiment, retinal reperfusion was observed when hyaluronidase was administered up to 10 minutes after occlusion. As a result, we can assume that retrobulbar hyaluronidase would be effective until 10 minutes after iatrogenic blindness by hyaluronic acid filler injection. Previous studies have failed when injecting 1000 IU of retrobulbar hyaluronidase at 30 minutes after obstruction.[7] Although the dose and administration time of hyaluronidase were different between the above experiments, both studies suggest that

hyaluronidase should be given in high doses or within at least 10 minutes to have a positive effect. Therefore, the situation can be reversed by administering it as soon as possible.

When a patient complains of visual disturbance or ocular pain after filler injection, the doctor should immediately check for direct and indirect pupil reflex and could consider high-dose retrobulbar hyaluronidase. A previous study suggested having an emergency kit and showed one case of right eye pain after injection of platelet-rich plasma.[11] Ophthalmologists have been using hyaluronidase as a spreading factor for retrobulbar or peribulbar regional blocks. With proper training, physicians should be able to use this fast procedure as rescue treatment for patients with impending vision loss caused by hyaluronic acid filler obstruction. In case of a vascular obstruction, transfer to the ophthalmologic center may be considered; however, delayed injection of hyaluronidase may lead to missing the best window of opportunity for treatment.

Hyaluronidase Dosage

In the Republic of Korea, more than 20 products of hyaluronidase are available, most of which are powdered, such as Liporase (Daehan New Pharm. Co., Hwasung, Republic of Korea), and are also available in liquid form, such as Hylex (BMI Korea Co., Ltd., Jeju, Republic of Korea). Approximately 1500 IU of hyaluronidase is mainly distributed. In the United States, Vitrase (Bausch Health Companies, Inc., Laval, Quebec, Canada) is extracted from ovine testicles and is available in 200 USP Units/ml. Hylenex (Halozyme Therapeutics, Inc., San Diego, Calif.) is produced by genetically manipulating human recombinant DNA in Chinese hamster ovary cells and is available in 150 USP Units/ml. To date, the literature recommends using approximately three to four hyaluronidase bottles, which is equivalent to only 450 to 600 IU of hyaluronidase and is not as high as in this experiment. Recent study suggests protocols of high-dose hyaluronidase to promote complete degradation of the hyaluronic acid filler.[12] We can hypothesize that using high doses of retrobulbar hyaluronidase in humans can also be feasible for treating iatrogenic blindness caused by fillers. Further experimentation should be conducted to determine the dosage of retrobulbar hyaluronidase injection.

CONCLUSIONS

Ocular damage is the most tragic complication of soft-tissue injection. In this rabbit model, 3000 IU of retrobulbar hyaluronidase was administered less than 10 minutes after occlusion to reverse obstruction or restore function following hyaluronic acid occlusion of the retinal branch ophthalmic artery.

Eun-Jung Yang, M.D., Ph.D.
Department of Plastic and Reconstructive Surgery
Hanyang University Seoul Hospital
Hanyang University, College of Medicine
222-1, Wangsimni-ro, Seongdong-gu
Seoul, Republic of Korea
enyang7@gmail.com

REFERENCES

1. Beleznay K, Carruthers JD, Humphrey S, Jones D. Avoiding and treating blindness from fillers: A review of the world literature. *Dermatol Surg.* 2015;41:1097–1117.
2. Carruthers J, Fagien S, Dolman P. Retro or peribulbar injection techniques to reverse visual loss after filler injections. *Dermatol Surg.* 2015;41(Suppl 1):S354–S357.
3. Wang M, Li W, Zhang Y, Tian W, Wang H. Comparison of intra-arterial and subcutaneous testicular hyaluronidase injection treatments and the vascular complications of hyaluronic acid filler. *Dermatol Surg.* 2017;43:246–254.
4. Zhu GZ, Sun ZS, Liao WX, et al. Efficacy of retrobulbar hyaluronidase injection for vision loss resulting from hyaluronic acid filler embolization. *Aesthet Surg J.* 2017;38:12–22.
5. Chesnut C. Restoration of visual loss with retrobulbar hyaluronidase injection after hyaluronic acid filler. *Dermatol Surg.* 2018;44:435–437.
6. Chiang C, Zhou S, Chen C, Ho DS, Zhang H, Liu K. Intravenous hyaluronidase with urokinase as treatment for rabbit retinal artery hyaluronic acid embolism. *Plast Reconstr Surg.* 2016;138:1221–1229.
7. Hwang CJ, Mustak H, Gupta AA, Ramos RM, Goldberg RA, Duckwiler GR. Role of retrobulbar hyaluronidase in filler-associated blindness: Evaluation of fundus perfusion and electroretinogram readings in an animal model. *Ophthalmic Plast Reconstr Surg.* 2019;35:33–37.
8. Michalinos A, Zogana S, Kotsiomitis E, Mazarakis A, Troupis T. Anatomy of the ophthalmic artery: A review concerning its modern surgical and clinical applications. *Anat Res Int.* 2015;2015:591961.
9. DeLorenzi C. Transarterial degradation of hyaluronic acid filler by hyaluronidase. *Dermatol Surg.* 2014;40:832–841.
10. Fagien S, Carruthers J. Commentary on restoration of visual loss with retrobulbar hyaluronidase injection after hyaluronic acid filler. *Dermatol Surg.* 2018;44:437–443.
11. Prado G, Rodríguez-Feliz J. Ocular pain and impending blindness during facial cosmetic injections: Is your office prepared? *Aesthetic Plast Surg.* 2017;41:199–203.
12. DeLorenzi C. New high dose pulsed hyaluronidase protocol for hyaluronic acid filler vascular adverse events. *Aesthet Surg J.* 2017;37:814–825.