Argatroban Inhibits Intraocular Fibrin Formation After Vitrectomy in Rabbits

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Objectives: To examine whether the specific thrombin inhibitor argatroban can prevent anterior chamber, pupillary area, and anterior vitreous fibrin formation after vitrectomy and lensectomy in rabbits.

Methods: Argatroban was infused into the vitreous cavities of Japanese albino rabbits for 5 minutes after pars plana vitrectomy and lensectomy. Slitlamp microscopy and indirect ophthalmoscopy were performed at postoperative hours 0.5, 1, 2, 3, and 6, and at postoperative days 1, 2, 3, and 7, and the amounts of fibrin formation in the anterior chamber, pupillary area, and anterior vitreous were scored from grade 0 to 4.

Results: Argatroban prevented fibrin formation from 0.5 hours postoperatively in a dose-dependent manner. In the eyes treated with 0.01% argatroban, the median score for postoperative fibrin formation was significantly less than that in control eyes between hours 1 and 3 (hour 1, P = .02; hour 2, P = .005; and hour 3, P = .003); the eyes treated with 0.003% argatroban also had significantly less fibrin than control eyes between 1 and 2 hours (hour 1, P = .005; hour 2, P = .03).

Conclusion: These results indicate that argatroban inhibits intraocular fibrin formation in an experimental rabbit model.

Clinical Relevance: Argatroban may be useful clinically in cases that often produce fibrin postoperatively, such as proliferative vitreoretinopathy and proliferative diabetic retinopathy.


In recent years, the treatment of severe proliferative vitreoretinopathy has met with increased success because of improvements in vitreoretinal instruments and techniques. However, in the treatment of severe proliferative vitreoretinopathy, fibrin occasionally develops in the anterior chamber, pupillary area, and anterior vitreous postoperatively, and may interfere with surgical success and visual outcome. Jaffe et al found that the factors predisposing to severe postoperative fibrin formation include severe flare, the presence of a previously placed scleral buckle, poor preoperative visual acuity, and intraoperative anterior epiretinal membrane dissection. Postoperative fibrin formation in the anterior segment may not only obstruct postoperative examination of the posterior segment, it may also contribute to pupillary block glaucoma and anterior proliferative vitreoretinopathy. Williams et al and Jaffe et al found that injection of recombinant tissue plasminogen activators into the anterior chamber or vitreous cavity resulted in complete resolution of postoperative fibrin. Systemic corticosteroids and anti-inflammatory drugs have also been used to prevent intraoperative damage to the blood-ocular barrier. Because fibrin is formed by conversion from fibrinogen by thrombin, Johnson and Blackenship suggested that intraocular heparin might prevent postoperative fibrin formation when performed as a prophylactic measure. However, heparin not only prevents fibrin formation by inhibitory effects on the heparin-antithrombin III complex, it also inhibits factors IX, X, XI, and XII in the blood-coagulation cascade. Therefore, heparin supplementation of the vitrectomy infusion might result in development of intraoperative and postoperative vitreous bleeding. Iverson et al proposed that low-molecular-weight heparins may be useful for prophylaxis of postoperative fibrin formation and for reducing hemorrhagic complications, as compared with normal heparin. Nevertheless, we believe that a more specific treatment to inhibit postoperative fibrin formation is necessary.

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SUBJECTS AND METHODS

Thirteen adult Japanese albino rabbits, weighing 1.5 to 2.0 kg, were anesthetized by intramuscular injection of ketamine hydrochloride (40 mg/kg) and xylazine hydrochloride (10 mg/kg). Pupils were dilated with 1% atropine sulfate, 0.5% tropicamide, and 0.5% phenylephrine hydrochloride eyedrops.

All procedures were performed in a sterile manner. A 360° peritomy was performed and the right eye was then dislocated anteriorly. A 20-gauge microvitrectoreal blade was used to make an inferonasal and superotemporal sclerotomies 2.0 mm posterior to the limbus. An infusion cannula was placed through the inferonasal sclerotomy and tied with 7-0 polyglactin suture.

During lensectomy and vitrectomy, Opeguard MA (Senju Pharmaceutical Co Ltd, Osaka, Japan) (glucose, 1.5 mg/mL; potassium chloride, 0.36 mg/mL; magnesium sulfate, 0.3 mg/mL; sodium chloride, 6.6 mg/mL; calcium chloride, 0.18 mg/mL; and sodium bicarbonate, 2.1 mg/mL) was irrigated through the infusion cannula. Lensectomy was performed by fragmentation for an average of 3.5 minutes. The lens cortex was aspirated completely with the vitreous cutter and the anterior lens capsule was left intact; vitrectomy was then performed. The infusion cannula was joined with a 3-way stopcock connected to the bottle of Opeguard MA, and supplemented with argatroban. Vitrectomy and lensectomy were performed under argatroban-free Opeguard MA irrigation, followed by irrigation of Opeguard MA fortified with argatroban. Argatroban-fortified Opeguard MA was infused at 40–mm Hg pressure for 5 minutes, while a superotemporal sclerotomy wound was left open. After 5 minutes of irrigation, the sclerotomy wounds were closed with 7-0 polyglactin suture.

The dislocated eye was replaced and ophthalmic antibiotic ointment was instilled into the operated-on eye.

The left eye was treated in the same manner as the right eye.

Each intravitreal infusion after vitrectomy and lensectomy was conducted in a masked fashion. Seven eyes had infusion of Opeguard MA with 0.01% argatroban; 10 had infusion of Opeguard MA with 0.003% argatroban; and 8 had infusion of Opeguard MA only (control group).

Postoperative slitlamp biomicroscopy and indirect ophthalmoscopy observations were performed at hours 0.5, 1, 2, 3, and 6 and days 1, 2, 3, and 7. Fibrin formation at the anterior chamber, pupillary area, and anterior vitreous was estimated by scoring with grades from 0 to 4 as follows: grade 0, no fibrin exudation; grade 1, slight fibrin exudation and thin fibrin membrane formation in the pupillary area; grade 2, fibrin membrane formation and partially fibrin clot in the pupillary area; grade 3, fibrin clot occupying the pupillary area; and grade 4, fibrin clot occupying the anterior chamber, pupillary area, and anterior vitreous. The postoperative slitlamp biomicroscopy photographs were evaluated in a masked fashion without knowledge of intravitreal infusion. Immediately after examinations by slitlamp biomicroscopy and indirect ophthalmoscopy on postoperative day 7, the rabbits were killed with an overdose of intravenous pentobarbital, and both eyes were immediately enucleated and fixed in 10% formalin in phosphate-buffered saline. The fixed globes were cut at the 12-o’clock meridian. Cross sections were cut at a thickness of 5 µm and stained with hematoxylin-eosin. Light microscopic examinations were performed to histopathologically evaluate the retinal damage in each eye.

All rabbits were treated in accordance with the Association for Research in Vision and Ophthalmology Statement for the Use of Animals in Ophthalmic and Vision Research. Postoperative fibrin formation was represented at the median score of grades at each point in each group. Statistical comparisons between groups were performed with the Mann-Whitney U test.

Our findings suggest that the specific thrombin inhibitor argatroban may be useful for prophylaxis of severe postoperative severe fibrin formation.

RESULTS

Fibrin formation could be observed on slitlamp biomicroscopy examination and estimated by scoring as early as postoperative hour 0.5. In the control group, postoperative fibrin formation tended to develop between hour 2 and hour 6 and to gradually decrease from day 1 to day 7. In contrast, in a dose-dependent manner, fibrin formation was reduced or retarded after 0.5 hours in the eyes treated with argatroban.

At hour 1, scores for fibrin formation in the groups treated with 0.01% and 0.003% argatroban were each 1. In contrast, the score for fibrin formation in the control group at hour 1 was 2. One control eye had grade 4 fibrin formation from hour 1 to day 1 (Figure 1). This severe fibrin clot decreased from day 2, but grade 1 fibrin formation was still present on day 7. The scores at hour 1 were significantly different between the 2 treated groups and the control group.
At hour 2, the median scores of fibrin formation in both the treated groups and the control group had not changed from those at hour 1. Scores were significantly different between the 2 treated groups and the control group at this time point (0.01%, $P=.005$; 0.003%, $P=.03$) (Figure 4).

At hour 3, the score of the group treated with 0.01% argatroban remained 1; however, 1 eye treated with 0.003% argatroban had progression to a score of 3 (Figures 1 and 2) (Figure 3, right). Scores of the group treated with 0.01% argatroban and the control group were significantly different ($P=.003$) (Figure 4). Furthermore, there was a significant difference between the groups treated with 0.01% argatroban and 0.003% argatroban ($P=.002$) (Figure 4).

After hour 6, the eyes treated with 0.01% argatroban tended not to have severe postoperative fibrin formation until day 3, although the difference in scores from the control group was not significant. During postoperative observations for 7 days, only 1 eye treated with 0.01% argatroban had grade 4 fibrin formation between hour 6 and day 3; however, the other 6 eyes continued to have less than grade 2 fibrin formation. On day 7, scores had decreased to 1 in all groups.

Ophthalmoscopically, only 1 eye treated with 0.003% argatroban had a mild vitreous hemorrhage at hour 0.5, which resolved by day 3. The source of this vitreous hemorrhage was not identified.

Histopathological examinations revealed no abnormality in any eye in any of the 3 groups.

**COMMENT**

Argatroban was developed as a specific thrombin inhibitor, and has a molecular weight of 526.66; it strongly inhibits thrombin. It has selectivity high enough that it does not inhibit plasmin even at a concentration 10000 times that required for inhibition of thrombin, since it binds...
very well to the active binding site on thrombin. Argatroban has been reported to inhibit thrombin from forming cross-linkages in fibrin to create a tough insoluble clot. Tamao et al\textsuperscript{14} has demonstrated concentration-dependent inhibition of fibrin cross-linking in fibrin isolated after 15 minutes.

In this study, in a dose-dependent manner, fibrin formation was reduced or retarded after hour 0.5 in the eyes treated with argatroban by intravitreal infusion. Despite the fact that argatroban has a half-life of 0.5 hours in blood, it could prevent postoperative fibrin formation by inhibiting thrombin in the blood coagulation cascade from postoperative hours 1 to 3, with significant differences in scores from the control eyes, and effectively until postoperative day 3.

The ratio of tissue plasminogen activator to total protein in normal human aqueous humor is about 30 times greater than the ratio of tissue plasminogen activator to total protein in plasma.\textsuperscript{16} The intraocular tissue plasminogen activator in aqueous humor is known to play an important role in dissolving intraocular fibrin formation. In the control group in this study, the intraocular tissue plasminogen activator might have played a role in dissolving and reducing postoperative fibrin formation from day 1. On the other hand, the fibrin degradation products that are formed by dissolving fibrin with plasmin and activated by tissue plasminogen activator exhibit chemotaxis, which contributes to continuous intraocular inflammation and more intraocular fibrin formation.\textsuperscript{17,\textsuperscript{18}} Thus, initial postoperative fibrin formation leads to further fibrin formation and contributes to severe postoperative fibrin membrane formation. We conclude that argatroban in intravitreal infusion during vitrectomy and lensectomy prevents initial postoperative fibrin formation for several hours postoperatively and inhibits formation of the fibrin degradation products that cause ensuing severe postoperative fibrin formation.

The shaving of vitreous base and anterior epiretinal membrane dissection contributes to the destruction of the blood-ocular barrier, which corresponds to tissue factor in the extrinsic system of the blood coagulation cascade. Because vitrectomy and lensectomy for severe proliferative vitreoretinopathy often require the anterior epiretinal membrane dissection, fibrin often forms postoperatively in the anterior chamber, pupillary area, and anterior vitreous. Breakdown of the blood-retinal barrier in proliferative diabetic retinopathy results in stimulation of the intrinsic system of the blood coagulation cascade. Therefore, the postoperative fibrin formation after vitrectomy for proliferative diabetic retinopathy, which contributes to activation of both the intrinsic and extrinsic systems of the blood coagulation cascade, occurs more readily than after vitrectomy for nondiabetic vitreoretinopathy.

At present, there is no effective way to prevent fibrin formation after vitrectomy for proliferative diabetic retinopathy. Systemic corticosteroids have undesirable effects on blood glucose control, and the injection of tissue plasminogen activator into the anterior chamber or vitreous cavity may be associated with complications of bleeding.\textsuperscript{19} Anticoagulative drugs also carry
the risk of unforeseen bleeding resulting from excessive inhibition of the multiple factors of the blood coagulation cascade. However, there was a significant difference between argatroban and heparin in effect on thrombin time and activated partial thromboplastin time, because argatroban is a specific thrombin inhibitor. Argatroban has less hemorrhagic potential than heparin. In this study, none of the eyes treated with argatroban had severe intraoperative or postoperative complications of bleeding, excluding slight vitreous bleeding in 1 eye. Therefore, we suggest that intravitreal infusion of argatroban may be useful in preventing postoperative fibrin formation in patients with proliferative diabetic retinopathy, but note that uncontrolled bleeding might occur if it is used in patients with active neovascularization.

Argatroban may have advantages over other specific thrombin inhibitors and heparin in inhibiting fibrin-bound thrombin because of its superior accessibility to the clot-fluid interphase, since argatroban has very low molecular weight and directly interacts with the active binding site of thrombin. We believe that the advantages of argatroban make it a potentially ideal agent for prophylaxis of postoperative fibrin formation after vitrectomy. Although light microscopy revealed no retinal abnormalities after argatroban infusion, additional studies, including electoretinographic testing and electron microscopy of histopathologic sections, are needed to determine whether argatroban is toxic to the eye.

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