Effect of Bevacizumab on Strabismus Surgery in Rabbits

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PURPOSE. This study was conducted to evaluate the effect of bevacizumab on postoperative inflammation and adhesion after strabismus surgery in rabbits.

METHODS. Fifteen New Zealand White rabbits were used for this study. Both eyes of each of 15 rabbits underwent reinsertion of the superior rectus muscle (SRM). The right eye of each animal received a subconjunctival bevacizumab injection (2.5 mg/0.1 mL). As controls, normal saline was injected subconjunctivally in the contralateral eye. To assess acute inflammation changes, macrophages, neutrophils, and monocytes were localized in the SRM using an anti-CD11b antibody at postoperative day 1. At 4 weeks, the sites of muscle reattachment were evaluated grossly for postoperative adhesion score and histologically for collagen formation.

RESULTS. Infiltration of acute inflammatory cells showing CD11b+ was significantly reduced in the bevacizumab injection group (P = 0.001). The difference in adhesion (SRM/conjunctiva and SRM/sclera) scores between the two groups was statistically insignificant (P = 0.93 and P = 0.85). Histopathologic findings revealed that muscle changes and fibrosis showed no significant difference (P = 0.69) between the treated eyes and the control eyes.

CONCLUSIONS. The intraoperative use of bevacizumab reduced inflammatory cell infiltration in the early stage of the procedure, but it was insufficient to prevent postoperative adhesion in rabbit eyes after extraocular muscle surgery. (Invest Ophtalmol Vis Sci. 2010;51:4585–4588) DOI:10.1167/iovs.09-5066

One of the most serious complications after strabismus surgery is the formation of adhesions. Postoperative adhesion and tissue scarring may result in unsatisfactory, unpredictable surgical outcomes and ocular motility dysfunction, particularly in cases that involve complicated operations. These adhesions may originate because of improper tissue handling, excessive bleeding or cauterization, extrusion of orbital fat into the surgical field, reaction to suture materials or implant materials, or postoperative infections.1–3

Managing these adhesions is often difficult. Surgical adhesiolyis and repositioning of extraocular muscle is not effective because existing adhesions may reform or new adhesions may appear. Several types of barrier materials between the involved structures have been used to prevent the reformation of adhesions but have shown variable success.5–8 Their use is limited because of complications such as infection or extrusion of implant. Results in the anti-postoperative effect of sodium hyaluronate have been conflicting.9,10 Although experimental studies with the antimitotics mitomycin C and 5-fluorouracil have shown positive results in decreasing adhesion formation,11,12 these agents may cause serious side effects, such as corneoscleral melt and anterior ischemia.13,14

Bevacizumab (Avastin; Genentech Inc., San Francisco, CA) is a complete humanized monoclonal antibody directed against all isoforms of vascular endothelial growth factor (VEGF). Bevacizumab was originally developed as a treatment for metastatic colorectal cancer,15 and it has been successfully applied for choroidal neovascular disorders including age-related macular degeneration as an off-label use.16 Given that several studies have shown bevacizumab to be a potent inhibitor of inflammatory tissue angiogenesis and lymphangiogenesis, it was suggested that bevacizumab may be used for an adjuvant to reduce postoperative inflammation and subsequent fibrosis.17,18 Recently, bevacizumab was also shown to effectively prolong bleb survival in glaucoma filtering surgery by reducing inflammation and fibrosis.19

VEGF was reported to locate to extraocular muscle and retro orbital tissue.20 Therefore, bevacizumab would be a viable option for controlling the inflammatory process and preventing the subsequent fibrosis after extraocular muscle surgery. We investigated the effect of bevacizumab on postoperative adhesions after strabismus surgery in the rabbit model.

METHODS

Fifteen adult New Zealand White rabbits (weight range, 2–3 kg; age, 20 weeks) were used for this study. All rabbits were confirmed to be free of ocular disease. All animals were handled in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

Surgical Procedures

All the operations were performed identically in both the experimental and the control group by one surgeon (JHJ) as described here. The rabbits were anesthetized with ketamine (400 mg/kg; Huons, Seoul, Korea) and xylazine (5 mg/kg; Bayer, Seoul, Korea), and each rabbit was placed in a stereotactic frame. Each lid was opened with a speculum, and bupivicaine was dropped in the conjunctival cul-de-sac. The superior conjunctiva and Tenon’s capsule were opened, and the superior rectus muscle (SRM) was isolated from the other tissues with the use of cotton swabs and a muscle hook. Two 6-0 Vicryl (polyglactin) sutures were placed close to the insertion site, and the SRM was disinserted from the globe. The muscle was reattached to its original insertion point by suturing at the sclera. The conjunctival peritomy was closed with interrupted 8-0 Vicryl sutures.

In the right (treated) eye of each rabbit, 2.5 mg (0.1 mL) bevacizumab was injected subconjunctivally, and 0.1 mL normal saline was injected subconjunctivally in the left (control) eye. The 30-gauge needle was inserted at the site of muscle reattachment, and the agent was injected slowly. The needle was removed 30 seconds after completion of the injection to allow the diffusion of bevacizumab into the subconjunctival space and to reduce leakage.

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After the operation, 3 mg/mL ofloxacin (Tarivid; Santen, Osaka, Japan) eyedrops was administered to each eye. All animals were observed and examined regularly by an investigator (HYC) in a masked fashion for evidence of gross inflammatory reaction and scar formation. The animals were randomly killed with overdoses of barbiturate anesthe sia either 1 day or 4 weeks after surgery, and all eyes were enucleated and examined.

**Acute Inflammation**

Acute inflammatory reaction after the muscle surgery was evaluated in five rabbits. Sites of muscle reattachment were isolated for histologic evaluation. Sections were stained with hematoxylin and eosin (H&E) for general histologic observation and with antibody for CD11b, which was specific for macrophages, monocytes, and neutrophils. The CD11b⁺ cell staining level was graded by a masked observer based on a histologic grading scale of 1 to 4 (grade 1, 0%–10%; grade 2, 10%–30%; grade 3, 30%–50%; grade 4, >50% CD11b⁺ cell density).

**Late Fibrosis**

Both eyes of 10 rabbits were evaluated for postoperative fibrosis. Gross adhesion between the muscle and the sclera and between the muscle and the conjunctiva were evaluated and recorded. Adhesion severities were scored from 0 to 3 according to the criteria in a previous report,⁴ where 0 indicated no adhesion, 1 indicated adhesion easily separated with blunt dissection, 2 indicated mild to moderate adhesion with a freely dissectible plane, and 3 indicated moderate to dense adhesion with difficult dissection or a nondissectible plane.

After the animals were killed, the sites of muscle reattachment were processed for histopathologic evaluation. Isolated tissues were examined using H&E stain for general histologic observation and with antibody for CD11b, which was specific for macrophages, monocytes, and neutrophils. The CD11b⁺ cell staining level was graded by a masked observer based on a histologic grading scale of 1 to 4 (grade 1, 0%–10%; grade 2, 10%–30%; grade 3, 30%–50%; grade 4, >50% CD11b⁺ cell density).

**Statistical Analysis**

Statistical analysis was performed to ascertain any differences in the acute inflammatory cell infiltration, postoperative adhesion score, and fibrosis score between the two groups using the Wilcoxon’s rank-sum test. Statistical significance was determined at $P < 0.05$.

**RESULTS**

The rabbits were monitored daily for changes within the orbit. All the rabbits appeared to be healthy and ate normally. Mild redness in the conjunctiva developed at the surgical site in some of the eyes. However, no significant ocular, periocular, or orbital changes were noted in the treated eyes, and no gross changes were visible in the treated muscles at necropsy. There was no evidence of systemic toxicity in any animal.

**Acute Inflammation**

Histologic examination using H&E staining showed inflammatory cell infiltration around SRM and surrounding tissue in both groups, but these inflammatory cell reactions were less prominent in the bevacizumab-treated group compared with the control group. One measure of acute inflammation was the influx of macrophages, neutrophils, and monocytes into the injured tissue. These cells could be visualized using the cell-specific antibody CD11b. There was a marked reduction in the density of CD11b⁺ cells in the bevacizumab-treated eyes compared with control eyes (Fig. 1; $P = 0.001$). The median grade and range of acute inflammatory cell density in both groups are listed in Table 1.

**Late Fibrosis**

Adhesion grades between SRM and the conjuctiva were as follows: bevacizumab injection group—1 in four eyes, 2 in five eyes, 3 in one eye; control group 4 weeks after surgery—1 in five eyes, 2 in four eyes, 3 in one eye. Adhesion grade between SRM and the sclera were as follows: bevacizumab injection group—0 in one eye, 1 in five eyes, 2 in four eyes; control group 4 weeks after surgery—0 in two eyes, 1 in four eyes, 2 in four eyes. As shown in Table 1, there was no significant difference in the severity of adhesion between the bevacizumab injection and the control group in the SRM/conjunctiva ($P = 0.93$) and the SRM/sclera ($P = 0.85$).

Histopathologic findings under light microscopes revealed granulomatous inflammation and fibrous connective tissue in both groups. There was no evidence of decreased scarring and granuloma formation in the bevacizumab-treated eyes compared with the control eyes (Fig. 2). There was loose and dense collagen deposition in connective tissue and mild fibrosis.

| TABLE 1. CD11b⁺ Cell Density in Each Group at 1 Day after Procedure, and Degree of Adhesion and Fibrosis in Each Group 4 Weeks after Surgery |
|---------------------------------|---------------------------------|-----------------|
| **Group 1**                     | **Group 2**                     | **$P^*$**       |
| **(Bevacizumab Injection)**     | **(Normal Saline Injection)**   |                 |
| CD 1b cell density              | CD 1b cell density              |                 |
| 1 (1–3)                         | 2 (1–4)                         | 0.001           |
| Degree of adhesion              | Degree of adhesion              |                 |
| SRM/conjunctiva                 | SRM/conjunctiva                 | 0.93            |
| 2 (1–3)                         | 1.5 (1–3)                       |                 |
| SRM/sclera                      | SRM/sclera                      | 0.85            |
| 1 (0–2)                         | 1 (0–2)                         |                 |
| Fibrosis                        | Fibrosis                        | 0.69            |
| 2.5 (1–5)                       | 2 (1–5)                         |                 |

Data are median (range: minimum score – maximum score). * Wilcoxon’s rank-sum test.

**FIGURE 1.** Cross-section through superior rectus muscle of rabbits stained for the presence of CD11b⁺ (arrow) 1 day after injection of (A) bevacizumab and (B) normal saline (CD11b immunohistochemical staining; original magnification, ×40). Note that there are fewer CD11b⁺ cells in the bevacizumab-treated eyes than in the control group.
within the muscle bundles themselves in both groups (Fig. 3). The fibrosis of the muscle and surrounding tissue was no different between the experimental and the control group (P = 0.69), which showed the same results, as confirmed by exploration.

**DISCUSSION**

Postoperative adhesive syndrome may develop secondary to strabismus surgery, retinal detachment surgery, and orbital traumas, causing damage to extraocular muscles and surrounding tissues. Because treatment of a preformed adhesion is difficult, it is important to prevent scarring during primary surgery. To achieve this purpose, many efforts have been made to reduce tissue trauma, bleeding, excessive cauterization, and postoperative inflammatory reactions and to alter the postoperative wound healing process. However, traumatic tissue injury, hemorrhage, and inflammation secondary to surgery are inevitable, and some amount of postoperative adhesion is a natural result.21

Efforts have been made to prevent the reformation of adhesion and to relieve restrictive motility dysfunction by various materials and pharmaceutical agents. Various types of material have been used to prevent postoperative scar formation, and these materials have included tissue implants such as Tenon’s capsule, peritoneum and plastic implants such as pig gelatin, polyethylene films, silicone, and specially designed plastic muscle capsules such as supramid muscle sleeves, polyyclatin mesh sleeves, and biomaterial mesh (Gore-Tex; W.L. Gore & Associates, Inc., Flagstaff, AZ).5–8 However, little clinical success has been reported with these materials. Foreign body reaction or extrusion has usually resulted from their use, thus defeating the purpose of their insertion.12

The results of the effect of sodium hyaluronate were contradictory. Searl et al.4 and Yaacobi et al.12 reported that sodium hyaluronate was a favorable result in strabismus surgery, whereas Fulga et al.9 reported that it was ineffective in the prevention of adhesion formation in a rabbit model. More recently, the antimitabolites and antifibroproliferative agent mitomycin C and 5-fluorouracil have been investigated and produced positive outcomes in reducing the formation of postoperative adhesions.11,12 However, these agents pose the risk for serious side effects such as corneal erosion and scleral melt,13 they have been known to decrease vascularity, and they are believed to increase the risk for anterior segment ischemia in patients who have undergone multiple strabismus surgery.12,14 Even though some reports state that subconjunctivally injected steroid produces cytotoxic effects on adjacent fibroblasts and that breakdown of the collagen fibers adds to reduced scar formation,22 steroidal agents may induce serious adverse effects, such as the elevation of intraocular pressure and the formation of cataract.

The initiation and control of inflammation is a complex process involving multiple steps. There is an immediate influx of neutrophils into the tissue, followed by the sequential invasion of macrophages into the connective tissue, where they ultimately surround the damaged muscle tissues.23 Another component of the inflammatory reaction is the development of vasodilatation and increased vascular permeability with resultant tissue edema.24 These inflammatory processes are followed by the activation and division of myofibroblasts and subconjunctival fibroblasts.25–27 These activated fibroblasts are related to adhesion and fibrosis.1,12

VEGF is a vital factor in the inflammatory process and the wound healing response.28–30 It is an important inducer of angiogenesis and a mediator of inflammatory cell migration and proliferation.30 Therefore, blocking the central role of VEGF in the inflammatory response would lead to a reduction in the inflammatory cell reaction and a decrease in scar formation.19,30 Successful outcomes have been reported that bevacizumab was effective in inflammatory ocular disease.17,18 and it would be a valuable option for antifibrosis and antiangiogenesis.30–32

In this study, there was a reduction in an early stage of inflammatory cell infiltration in a bevacizumab injection group compared with the control group; however, the postoperative adhesion score between the two groups did not differ signifi-
cantly. These results may be interpreted as an insufficient dose or a less effective route of bevacizumab delivery that could lead to only partial inhibition of fibroblast activity. However, other factors in addition to VEGF may be involved in the inflammatory process and the activation of fibroblasts in the extraocular muscle and surrounding connective tissue. Thus, we expect that combining the use of bevacizumab with other treatment modalities, such as mitomycin C or 5-fluorouracil, is likely important for improving postoperative scar prevention.

The effects of repeated exposure and the half-life of bevaciuzumab on the extraocular muscle and surrounding connective tissue are unknown, and bevacizumab on strabismus surgery is an uncontrolled procedure that would be related to the unpredictable outcomes. Therefore, further studies are required to evaluate the repeated use and long-term application of bevacizumab for extraocular muscle surgery.

In conclusion, though definite clarification for the mechanism of action requires further research, the results of our study demonstrate that bevacizumab was effective in reducing inflammatory cell infiltration in the early stage of the procedure and that it would be a viable option for reducing postoperative adhesions after extraocular muscle surgery.

References