Intravitreous Bevacizumab Injection

An Experimental Study in New Zealand White Rabbits

Rafael T. Cortez, MD; Gema Ramirez, MD; Lucienne Collet, MD; Pranjal Thakuria, MD; G. Paolo Giuliari, MD

Objectives: To determine the effects of intraocular pressure (IOP) and needle diameter on the amount of reflux after intravitreous bevacizumab injection.

Methods: Prospective randomized interventional study. Twelve New Zealand white rabbits weighing approximately 2.5 to 3.5 kg each were randomized 1:1 to group 1 or group 2. Bevacizumab stained with trypan blue was used for intravitreous injection. To lower the IOP, eyes in group 2 underwent anterior chamber paracentesis before intravitreous injection. Two eyes in each group were injected using 27-, 30-, or 32-gauge needles. If a subconjunctival bleb formed after intravitreous injection, its diameter was measured using a caliper.

Results: The median IOP in group 1 was 17.5 mm Hg. Eyes injected using 27-gauge and 30-gauge needles showed stained subconjunctival blebs with median sizes of 3 mm and 1.7 mm, respectively; eyes injected using 32-gauge needles showed no subconjunctival bleb formation. The median IOP in group 2 was 10.3 mm Hg. Eyes injected using 27-gauge needles showed stained subconjunctival blebs with a median size of 0.7 mm, and eyes injected using 30-gauge and 32-gauge needles showed no subconjunctival bleb formation.

Conclusion: Decreasing the IOP before intravitreous injection and using a smaller-gauge needle reduce the risk of drug reflux after intravitreous bevacizumab injection.

Clinical Relevance: Intravitreous injection is an increasingly common route of drug delivery to treat ocular diseases. Techniques that maximize bioavailability are examined in this study.

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Video available online at www.archophthalmol.com

VASCULAR ENDOTHELIAL growth factor (VEGF) acts in different physiologic processes, such as bone growth, tissue maintenance, wound healing, vasodilatation, and survival of various neuronal cell types, including retinal neurons. It has an active role in trophic maintenance of capillaries in several organs. In the eye, development of the choriocapillaris is dependent on continuous trophic support via VEGF secreted by the retinal pigment epithelium. The production of VEGF is increased when these cell types are subjected to hypoxia. In recent years, a strong association has been found between VEGF and development of ocular neovascular diseases.

Bevacizumab is a potent monoclonal antibody that blocks all VEGF isoforms. Bevacizumab was the first anti-VEGF therapy approved by the US Food and Drug Administration for the treatment of breast, lung, and colorectal cancer. After the success of preliminary investigations with ranibizumab (an agent similar to bevacizumab) in the treatment of age-related macular degeneration, researchers and clinicians were motivated to systemically and intravitreously use bevacizumab off label to treat age-related macular degeneration and other forms of choroidal neovascular membranes.

Because of the significant adverse effects associated with the use of systemic anti-VEGF medications, a trend to administer bevacizumab by intravitreous injection has been seen among vitreoretinal surgeons. Although some authors advocate the ocular safety of bevacizumab, it may enter the systemic circulation after the intravitreous route. Nevertheless, intravitreous injections of this agent provide an effective route for retinal neovascular disease therapy. A drawback of this technique is the risk of associated complications such as endophthalmitis, retinal...
detachment, and cataract formation. Recently, attention has been paid to other complications such as temporary intraocular pressure (IOP) increase and reflux of medication, with subconjunctival bleb formation after intraocular injection. Several ophthalmologists have modified the intravitreal injection technique in an effort to decrease the incidence of this reflux; however, most researchers have not considered the role of IOP.

The objectives of this study were to determine the effects of IOP and needle diameter on the amount of reflux after intravitreal bevacizumab injection. We also aimed to determine if bevacizumab is present in the subconjunctival bleb.

**METHODS**

**STUDY DESIGN**

This was a prospective randomized interventional study with direct comparison of the reflux after intravitreal bevacizumab injection and the effects of IOP and needle diameter on the amount of reflux, measured by subconjunctival bleb formation. The ethical committee of the Centro de Cirugía Oftalmológica and Universidad Central de Venezuela, Caracas, approved the study. All experiments were performed in accord with the research association for the use of animals at the Universidad Central de Venezuela.

**SUBJECT SELECTION AND RANDOMIZATION**

Twelve New Zealand white rabbits weighing approximately 2.5 to 3.5 kg each were obtained from the Animal Research Department of the Universidad Central de Venezuela. Rabbits were chosen for this study because of their usefulness in the evaluation of new drugs and surgical procedures for glaucoma. They were randomized 1:1 to group 1 or group 2. Eyes in group 1 were considered the control group, as no attempt was made to lower the IOP. In group 2, anterior chamber paracentesis was performed to lower the IOP. All study eyes included in each group (group 1 and group 2). In group 2, eyes were randomized 1:1:1 to group 1 or group 2. Eyes in group 1 were considered the control group, as no attempt was made to lower the IOP. In group 2, anterior chamber paracentesis was performed to lower the IOP. Two eyes in each group were then randomized 1:1:1 to receive intravitreal bevacizumab injections using 27-, 30-, or 32-gauge needles.

**TECHNIQUE AND TREATMENT**

Rabbits in both groups were anesthetized by a certified anesthesiologist using intramuscular ketamine hydrochloride injection. Topical anesthesia with proparacaine hydrochloride, 0.5%, was administered to each study eye 1 to 5 minutes before intravitreal injection. The intravitreal injection solution consisted of a mixture of 0.8 mL of bevacizumab and 0.2 mL of trypan blue. Trypan blue was used to stain the bevacizumab and to determine its presence if a subconjunctival bleb formed. The external area was observed for the presence of any subconjunctival bleb that stained blue. If present, the subconjunctival bleb was measured using a straight Castroviejo caliper (K3-9000; Katena Products, Inc, Denville, New Jersey).

Twelve eyes of 12 New Zealand white rabbits were included in the study. After randomization, 6 eyes were included in each group (group 1 and group 2). In group 2 eyes, the IOP was lowered by anterior chamber paracentesis using the aforedescribed technique. All study eyes were injected with the bevacizumab–trypan blue mixture. After the second randomization, 2 eyes in each group were injected using 27-, 30-, or 32-gauge needles.

**GROUP 1**

Eyes in group 1 had a median IOP of 17.5 mm Hg (range, 17-18 mm Hg). Eyes injected using 27-gauge needles showed trypan blue–stained subconjunctival blebs with a median size of 3 mm (range, 2.9-3.1 mm) (Figure, A). Eyes injected using 30-gauge needles showed trypan blue–stained subconjunctival blebs with a median size of 1.7 mm (range, 1.6-1.8 mm). Eyes injected using 32-gauge needles showed no subconjunctival bleb formation.

**GROUP 2**

To lower the IOP, eyes in group 2 underwent anterior chamber paracentesis before intravitreal injection. After this procedure, eyes in group 2 had a median IOP of 10.3 mm Hg (range, 10-11 mm Hg). Eyes injected using 27-gauge needles showed trypan blue–stained subconjunctival blebs with a median size of 0.7 mm. Eyes injected using 30- or 32-gauge needles showed no subconjunctival bleb formation (Figure, B).

**STATISTICAL ANALYSIS**

Comparing the effects of 32-gauge vs 30-gauge and 27-gauge needles in group 1, the median size of subconjunctival bleb was measured using a straight Castroviejo caliper (K3-9000; Katena Products, Inc, Denville, New Jersey).
The effects of 32-gauge and 30-gauge vs 27-gauge needles were compared in group 2. The median size of subconjunctival blebs differed significantly (P < .001). Comparing the effects of 27-gauge needles in group 1 vs group 2, the median size of subconjunctival blebs differed significantly (P = .002). Comparing the effects of 30-gauge needles in group 1 vs group 2, the median size of subconjunctival blebs also differed significantly (P = .003).

In conclusion, we observed in our cohort of eyes that subconjunctival blebs formed after intravitreous injection contain bevacizumab instead of fluid vitreous humor alone. In addition, the size of subconjunctival blebs is in direct proportion to the IOP and the needle diameter. Limitations of our study include our small sample size, as well as reported IOP measurement variation in New Zealand white rabbits. Until larger prospective randomized interventional studies are performed, we recommend decreasing the IOP before intravitreous injection and using a smaller-gauge needle to reduce the amount of drug reflux after intravitreous bevacizumab injection.

COMMENT

In 1911, Ohm introduced the use of intravitreous injections of air to repair retinal detachment. In the 1940s, intravitreous drug injections provide an effective route for retinal disease therapy. Since the advent of anti-VEGF therapies, use of the intravitreous injection technique has steadily increased. However, concern has been expressed about potential unwanted systemic absorption of these drugs. The effects may lead to serious complications such as systemic hypertension, thromboembolic diseases, and death.

A 2007 study evaluated short-term IOP after intravitreous bevacizumab injection. The authors reported IOP elevation 30 minutes after intravitreous injection in a few patients. These results have been confirmed by others in 2 studies. In one study, patients required eyedrops to lower the IOP; however, no patients needed anterior chamber paracentesis. In the other study, IO of less than 30 mm Hg was seen 15 minutes after intravitreous injection in all patients, also without need for anterior chamber paracentesis. Nevertheless, a point of concern is that reflux may occur after intravitreous bevacizumab injection following removal of the needle, causing a subconjunctival bleb that may contain some of the injected drug, which might affect drug bioavailability and absorption. To minimize the amount of vitreous reflux, a modified technique using a tunneled scleral incision has been suggested. Anterior chamber paracentesis before intravitreous injection may prevent reflux, ensuring that the complete dose of the agent used remains in the vitreous cavity; however, anterior chamber paracentesis per se carries the risks of infection and lens damage.

While injecting intravitreous bevacizumab in our practice, we observed that the drug inside the eye has an oily appearance. It adheres to the tip of the needle and is “pulled” to the vitreous base when withdrawing the needle (video; http://www.archophthalmol.com).

Herein, we considered not only the effects of needle diameter and an oblique injection technique as suggested by previous authors but also the possible key role of IOP in reflux after intravitreous injection. Our results showed that decreasing the IOP before intravitreous injection and using a smaller-gauge needle reduce the amount of drug reflux after intravitreous bevacizumab injection.

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