Intravitreous Bevacizumab Injection

An Experimental Study in New Zealand White Rabbits

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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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detachment, and cataract formation.23 Recently, attention has been paid to other complications such as temporary intraocular pressure (IOP) increase and reflux of medication, with subconjunctival bleb formation after intravitreous injection.24-26 Several ophthalmologists have modified the intravitreous injection technique in an effort to decrease the incidence of this reflux; however, most researchers have not considered the role of IOP.27,28

The objectives of this study were to determine the effects of IOP and needle diameter on the amount of reflux after intravitreous 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 intravitreous 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 Cirugia Oftalmologica 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.29,30 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.31,32 Topical anesthesia with proparacaine hydrochloride, 0.5%, was administered to each study eye 1 to 5 minutes before intravitreous injection. The intravitreous 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 in- terocular surface and cul-de-sac were rinsed generously with a povidone-iodine solution, 5%, while the eyelids were scrubbed with a cotton-tipped applicator soaked in povidone-iodine solution, 10%. After placing a sterile eyelid speculum, povidone-iodine solution, 5%, was applied directly over the injection site. Using a 30-gauge needle, eyes in group B underwent anterior chamber paracentesis in a controlled manner under magnification before intravitreous injection.33 The volume extracted by anterior chamber paracentesis varied from approximately 100 to 200 μL. Intracu- lar pressure was then assessed (Tono-Pen XL; Medtronic Solan, Jacksonville, Florida) in eyes of both groups. All intravitreous injections were performed by one of us (R.T.C.) with retina training and experience using an oblique intravitreous injection technique delivered 1.5 to 2 mm posterior to the superotemporal limbus. Using a 27-, 30-, or 32-gauge needle, 0.025 mL of the previously described bevacizumab–trypan blue mixture was injected. As soon as the needle was withdrawn, 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).

STUDY END POINTS

The primary end point was to determine if the IOP had an effect on the amount of reflux after intravitreous bevacizumab. Secondary end points were to determine if the needle diameter had a role in subconjunctival bleb formation and whether the content of the subconjunctival bleb was composed of refluxed bevacizumab.

STATISTICAL ANALYSIS

The results obtained in group 1 and group 2 were compared. Statistical analysis was performed using unpaired t test and commercially available software (STATA 8; StataCorp LP, College Station, Texas).

RESULTS

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 aforesaid 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). 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 intravitreous 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.

STATISTICAL ANALYSIS

Comparing the effects of 32-gauge vs 30-gauge and 27-gauge needles in group 1, the median size of subcon-
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travitreous 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.32

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 authors27,28 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.

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.32 Until larger prospective randomized interventional studies are performed, we recommend decreasing the IOP before intravitreous bevacizumab injection and using a 32-gauge needle and an oblique injection technique. This technique includes placement of a cotton swab at the injection point immediately after removal of the needle in an effort to avoid reflux of bevacizumab, which may enter the systemic circulation. Intraocular pressure can be reduced by anterior chamber paracentesis.32 However, in our practice we prefer to place a mercury bag over the eye for 20 to 30 minutes before intravitreous injection. This is effective and avoids the risks associated with anterior chamber paracentesis.

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