Video Article

Intravitreous Injection for Establishing Ocular Diseases Model

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Abstract

Intravitreous injection is a widely used technique in visual sciences research. It can be used to establish animal models with ocular diseases or as direct application of local treatment. This video introduces how to use simple and inexpensive tools to finish the intravitreous injection procedure. Use of a 1 ml syringe, instead of a hemilton syringe, is used. Practical tips for how to make appropriate injection needles using glass pipettes with perfect tips, and how to easily connect the syringe needle with the glass pipette tightly together, are given.

To conduct a good intravitreous injection, there are three aspects to be observed: 1) injection site should not disrupt retina structure; 2) bleeding should be avoided to reduce the risk of infection; 3) lens should be untouched to avoid traumatic cataract. In brief, the most important point is to reduce the interruption of normal ocular structure. To avoid interruption of retina, the superior nasal region of rat eye was chosen. Also, the puncture point of the needle was at the par planar, which was about 1.5 mm from the limbal region of the rat eye. A small amount of vitreous is gently pushed out through the puncture hole to reduce the intraocular pressure before injection. With the 45° injection angle, it is less likely to cause traumatic cataract in the rat eye, thus avoiding related complications and influence from lenticular factors. In this operation, there was no cutting of the conjunctiva and ocular muscle, no bleeding. With quick and minor injury, a successful intravitreous injection can be done in minutes.

The injection set outlined in this particular protocol is specific for intravitreous injection. However, the methods and materials presented here can also be used for other injection procedures in drug delivery to the brain, spinal cord or other organs in small mammals.

Protocol

1. Prepare the glass pipettes using pipettes puller, connect it to a 1ml syringe, and seal the connection with parafilm. Withdraw 2 ml of solution into the tip of the pipettes and get ready for injection.
2. Anaesthetize the rat by intra-peritoneal injection of ketamine (80mg/kg) and xylazine (8mg/kg) (volume ratio at 2:1).
3. Apply one drop of 0.5% alcaine to the rat eyes as topical anesthetics before intravitreous injection.
4. Position the rat and expose the superior nasal region of the eye.
5. Use a 30 gauge needle to puncture the superior nasal sclera at the level of the par plana. Avoid touching the ocular muscle and vessels.
6. Give the eye ball mild pressure to get rid of a small amount of vitreous through the puncture hole from the posterior chamber.
7. Insert the tip of the glass pipette through the puncture hole at a 45° angle through the sclera into the vitreous body. Inject 2 ml of solution into the posterior chamber and keep in place for about a few seconds, then gently remove the needle. Avoid touching the lens and intraocular vessels.
8. After intravitreous injection, apply ophthalmic Tobrex ointment on the rat eye to prevent infection.

Disclosures

The animals were handled according to the protocol for the use of animal in research approved by the Committee on the Use of Live Animals in Teaching and Research (CULATR) of the University of Hong Kong and the Association for Research in Vision and Ophthalmology Statements for the use of animals in Ophthalmic and Vision Research.

Discussion

Good intravitreous injection is useful for establishing animal model as well as direct intraocular treatment. First, injection site should not disrupt retina structure. Second, bleeding should be avoided to reduce the risk of infection. Third, lens should be untouched to avoid traumatic cataract. Most important is to reduce the interruption of normal ocular structure.

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