Intravitreous administration in the rabbit and minipig

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Introduction

Intravitreous administration to the eye brings drugs into direct access with the retina, and there has been much recent interest in the clinical use of this route of administration in the therapy of macular degeneration and other diseases. For the preclinical development of these approaches, intravitreous administration to laboratory animals is required.

Materials and methods

A total of 252 New Zealand White rabbits (2 to 4 month old), 112 Dutch Belted rabbits (3 to 12 month old) and 223 Göttingen minipigs (3 to 6 month old) were used for toxicity studies by single or repeated (up to 9 weekly) intravitreal administrations.

Injections were performed with the animal under ketamine (Imalgene™) general anesthesia. Local analgesia was achieved by corneal instillation of tetracaine 1% and mydriasis was obtained by topical instillation of the antimuscarinic agent tropicamide (Mydriaticum™). The eyes were flushed with povidone-iodine (Betadine™ 2%) for prophylactic local antisepsy.

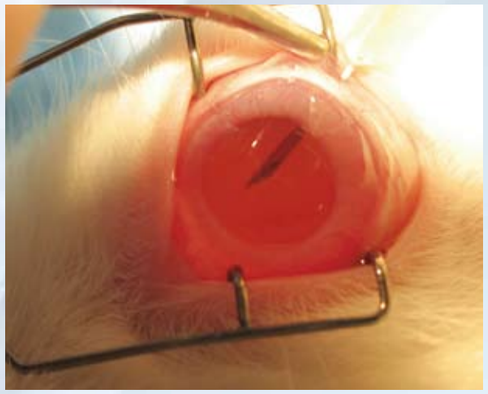

Figure 1: Needle inserted into the vitreous of a rabbit eye.

Eyelids were retracted with a pediatric Barraquer wire speculum and the ocular scleral conjunctiva was grasped at the lateral canthus with a Von Graefe forceps to laterally rotate the globe towards the medial canthus. The needle tip, with its bevel directed backward, was positioned on the lateral sclera 4 to 5 mm posterior to the limbus. The eye was replaced to its natural position and the needle was directed backward with an approximate angle of 45° and pushed through the sclera to c.a. 10 mm into the posterior ocular chamber towards the center of the vitreous (figure 1). Volumes of 50 to 100 µL were injected. The needle was slowly withdrawn after the injection and the scleral conjunctiva was slightly compressed with the forceps tip to avoid any release of the injected material.

Slit lamp biomicroscopy of the anterior ocular segment and fundus examination by indirect ophthalmoscopy were performed before and after each injection and at weekly intervals. Direct and consensual pupillary light reflexes were recorded at the same occasions. Ganzfield electroretinograms were recorded under photopic and scotopic (after dark adaptation) conditions before the first administration and between 2 days and 39 weeks after the last injection.

At necropsy, between 2 days and 39 weeks after the last injection, all eyes were sampled and fixed in modified Davidson's fixative. Histologic examinations were conducted on vertical sagittal and parasagittal sections stained with hemalun eosin..

Results

A total of 1837 and 764 intravitreal injections were performed respectively in rabbits and minipigs. Besides proprietary excipients which were used as placebo, isotonic solutions of sodium chloride (NaCl) and phosphate buffer (PBS) solutions were used as controls. Isotonic NaCl was injected 112 times to 62 rabbit eyes and 62 times to 32 minipig eyes. PBS injections cumulated to 26 in 26 rabbit eyes and 28 in 28 minipig eyes.

The incidence of findings during ophthalmologic examinations was very low in the animals receiving isotonic NaCl solution, and almost none were observed in animals given PBS (Tables I and II). Droplets were observed in the vitreous body just after injection at a much higher frequency with isotonic NaCl (1 out of 6 injections in rabbits and 1 out of 6 injections in minipigs) than with PBS (1/26 injection only in rabbits) and were no more observed 1 week later. In rare cases conjunctival hyperemia or chemosis and blood extravasation into the vitreous were observed after injection and resolved within one week. Other findings were related to ocular inflammation and mainly consisted in white/grey area or strands which sometimes blurred or masked the observation of the eye fundus, and usually regressed within weeks. Iritis with myosis were only observed in 4 minipigs and evolved to loss of pupillary light reflex in 3 animals. No modifications of the ERG were observed in the 62 isotonic NaCl- treated rabbit eyes, and decreased of a and/or b wave amplitudes were noted in 6/32 minipig eyes, including those 3 with loss of pupillary reflexes, injected with isotonic NaCl.

Finding	Isotonic NaCl	PBS
Conjunctival hyperhemia/ Chemosis	-	2
Droplets in the vitreous body	10	1
White/grey area in the vitreous	5	-
Blood extravasation into the vitreous	2	-
Total number of eyes (injections)	**62 (112)**	**26 (26)**

Table I: Treatment-related findings observed during ophthalmoscopic examinations in rabbits

Finding	Isotonic NaCl	PBS
Conjunctival hyperhemia	5	2
Myosis	4	1
Iritis	4	-
Droplets in the vitreous body	6	-
White/grey area in the vitreous	10	-
Blood extravasation into the vitreous	1	-
Loss of direct pupillary light reflexes	3	-
Total number of eyes (injections)	**32 (62)**	**28 (28)**

Table II: Treatment-related findings observed during ophthalmoscopic examinations in minipigs

Finding	Isotonic NaCl			PBS		
Grade of the lesion	*1*	*2*	*>3*	*1*	*2*	*>3*
Bulbar conjuctiva: mixed inflammatory cell infiltrate	15	3	-	1	-	-
Vitreous body: Inflammatory cells	-	-	-	1	-	-
Retinal: folds	2	-	-	-	-	-
Total number of eyes (injections)	**52 (112)**			**26 (26)**		

Table III: Treatment-related findings observed during histologic examinations in rabbits

Finding	Isotonic NaCl			PBS		
Grade of the lesion	*1*	*2*	*>3*	*1*	*2*	*>3*
Bulbar conjuctiva: mixed inflammatory cell infiltrate	16	4	-	1	-	-
Iris or ciliary bodies: inflammatory cell infiltrateinfiltrate	5	4	-	-	-	-
Lens: degeneration	1	-	1	-	-	-
Vitreous body: Inflammatory cells	3	3	4	-	-	-
Vitreous body: fibroplasia	-	2	2	-	-	-
Retina: perivascular cuffs of inflammatory cells	7	3	-	-	-	-
Retina: cell layer loss	1	-	1	-	-	-
Retinal detachment	-	-	3	-	-	-
Total number of eyes (injections)	**32 (62)**			**28 (28)**		

Table IV: Treatment-related findings observed during histologic examinations in minipigs

No macroscopic findings were recorded at necropsy; however there was a good correlation in nature and incidence of histopathological and ophthalmological findings (Tables III and IV). These later consisted in few minor-grade (1 or 2) inflammatory lesions and exceptional inflammatory and degenerative lesions of grade equal or higher than 3, which were mainly noted as a pan-ocular inflammation in 3 minipigs correlating with the loss of papillary light reflexes and disappearance of ERG waves. The observation of retinal folds or retinal detachment was exceptional in minipigs, correlated with the disappearance of ERG waves, and observed in the context of severe inflammation of the ocular globe. The incidence and severity of lesions in eyes injected with PBS appear to be lower than those observed in eyes given isotonic NaCl.

There was no difference in the incidence and severity of ophthalmologic and histopathologic findings when 50 or 100 µL were selected as injection volumes in rabbits or minipigs. In both species, PBS induced much less ophthalmologic findings or histologic lesions than isotonic NaCl. Most ocular globes of rabbits or minipigs injected with saline solutions appeared normal at histological examination (figures 2 and 3).

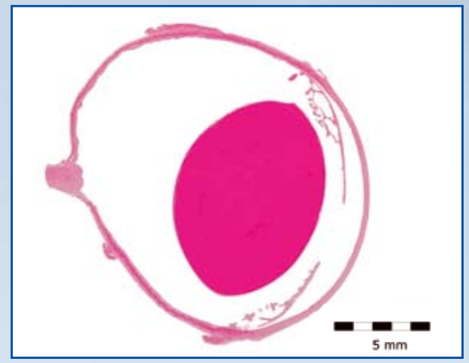

Figure 2: Histological appearance of a rabbit eye after intravitreous injection of saline solution

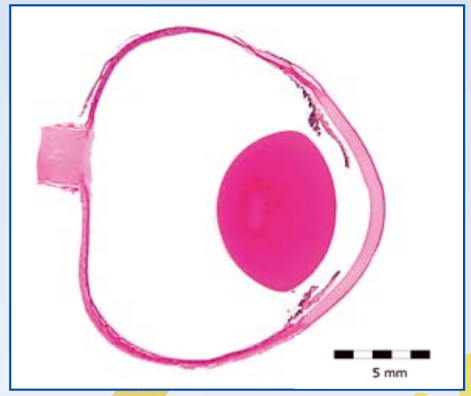

Figure 3: Histological appearance of a minipig eye after intravitreous injection of saline solution

Conclusion

The control of critical points in the intravitreal administration technique, and especially adequate preparation of the animals, appropriate volume and rate of administration, needle gauge and sterile technique, are key to the clinical tolerance of this procedure. This was clearly demonstrated at CIT, where a total of 228 intravitreal injections of 50 or 100 µL of isotonic saline solutions to rabbits or minipigs only resulted in 3 cases (1.3%) of severe ocular globe inflammation and a total of 6 cases (6.4%) of disappearance of ERG waves out of 94 isotonic NaCl injected eyes. Notably, PBS appears much better tolerated than isotonic NaCl solution both in rabbits and minipigs.