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Evaluation of Formulation pH Tolerability in New Zealand White and Dutch Belted Rabbits Post-Intravitreal Administration

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INTRODUCTION

This study investigates the tolerability of acidic intravitreal formulations in New Zealand White (NZW) and Dutch Belted (DB) rabbits over a 57-day period.

Developing intravitreal drugs presents challenges due to the eye's sensitivity and limited safe injection volume, often necessitating pH adjustments outside physiological levels[1,2].

While pH levels between 5.5 and 7.4 are generally well tolerated, concerns persist regarding more acidic solutions[3].

Our purpose is to evaluate the tolerability of formulations with pH levels of 4.0, 5.0, and 7.0 following a single intravitreal injection in both eyes.

METHODS

A total of 18 rabbits (6 males/3 females NZW and 6 males/3 females DB), ranging in age from 4 to 18 months, were divided into three groups:

- Group 1: vehicle control (150 mM Sodium Chloride, pH 7.0)
- Group 2: Acetate Chloride (20 mM Acetate, 150 mM Sodium Chloride, pH 4.0)
- Group 3: Acetate Chloride (20 mM Acetate, 150 mM Sodium Chloride, pH 5.0)

A single bilateral intravitreal injection of 100 µL was administered, followed by an 8-week observation period.

Ocular assessments included weekly Draize scoring, weekly intraocular pressure (IOP) measurements, ophthalmic examinations including slit lamp biomicroscopy and indirect ophthalmoscope, and fundus imaging at Weeks 1, 2, 4, 6, and 8.

Electroretinography (ERG) and Confocal Scanning Laser Ophthalmoscopy/ Optical Coherence Tomography (cSLO/OCT) imaging were performed at baseline, Week 4, and Week 8. The cSLO images were taken using the near-infrared (IR), and the sdOCT images were acquired as raster scans (9 automatic real-time tracking [ART]) or single scans (21 ART).

At termination, eyes were collected and fixed in Davidson's fixative and sent to StageBio for histological evaluation.

At StageBio: Eyes were sectioned serially (50 µm apart) using in-life images to guide the sectioning through the area(s) with identified lesions. For each level, 4-5 µm thickness sections were generated and stained with Hematoxylin and Eosin (H&E).

Table 1. Study Experimental Design

Group	Test material	pH	Osmolarity (mOsm/kg)	Dose volume (µL/eye)	Main	
					Males/breed	Females/breed
1	Vehicle control (150 mM sodium chloride)	7.0	282	100	2 NZW and 2 DB	1 NZW and 1 DB
2	20 mM acetate, (150 mM sodium chloride)	4.0	346	100	2 NZW and 2 DB	1 NZW and 1 DB
3	20 mM acetate, (150 mM sodium chloride)	5.0	338	100	2 NZW and 2 DB	1 NZW and 1 DB

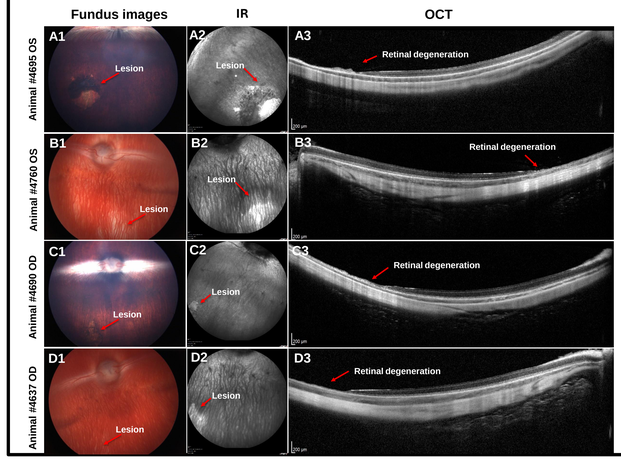


Figure 1. Representative *in vivo* images from animals from Group 2 show alterations in retinal morphology

Representative fundus images from four cases exhibiting retinal abnormalities are presented as follows:

- A1, B1, C1, D1: Fundus color images displaying round, discolored lesions.
- A2, B2, C2, D2: IR cSLO images highlighting the lesions as regions of discoloration in the inferior retina.
- A3, B3, C3, D3: OCT scans revealing retinal degradation, with red arrows marking corresponding areas in the fundus color and IR images.

These images collectively demonstrate the morphological changes observed in the retina across different imaging modalities, facilitating a comprehensive assessment of retinal pathology.

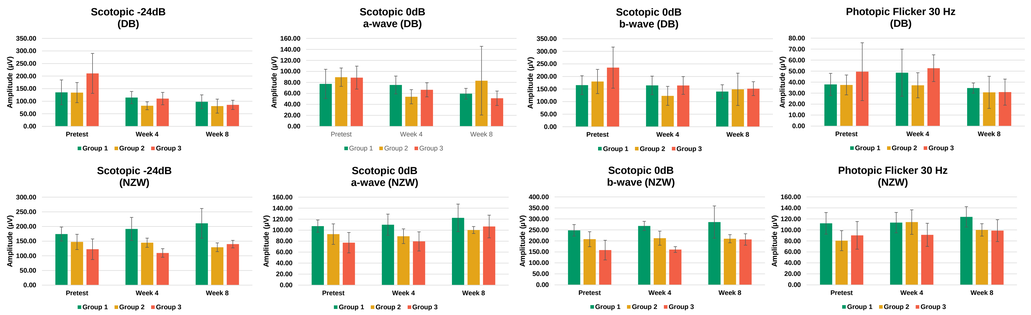


Figure 2. ERG Response: Scotopic and Photopic Amplitudes Over Time

RESULTS

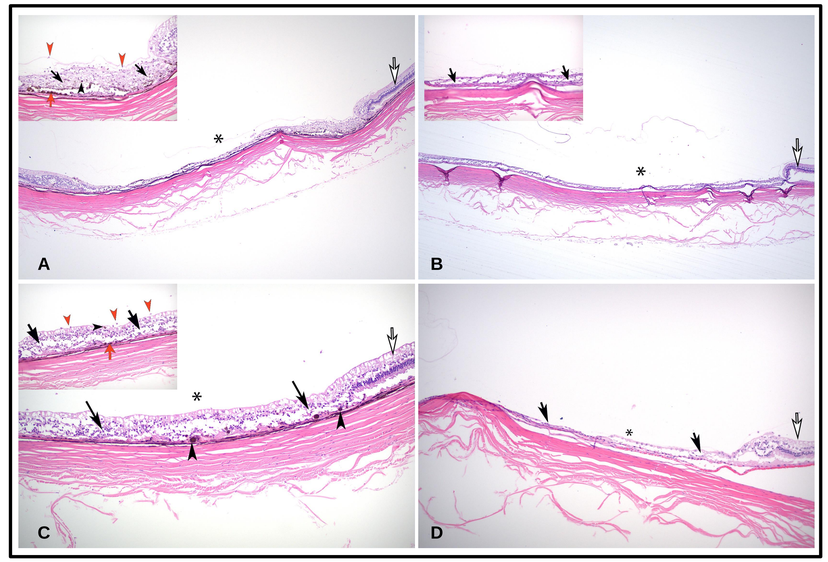
Intolerance to low pH 4.0 (Group 2) was observed as focal retinal degeneration in 7 of 12 eyes as early as Day 12 (Week 2) by ophthalmic examination and fundus imaging and confirmed by cSLO/OCT evaluation at Week 4. At Day 57, focal retinal degeneration/atrophy was confirmed in the 7 of 12 eyes treated with low pH 4.0 (Group 2) test material. Additional findings associated with the focal retinal degeneration/atrophy included minimal hypertrophied retinal pigment epithelium, minimal vitreal mononuclear cell infiltrates, and rare pigmented cells (macrophages and/or RPE) within the retina (DB rabbits only).

No in-life findings were observed in any animals from Groups 1 (vehicle control) and 3 (Acetate Chloride, pH 5.0) by ophthalmologic evaluations, fundus imaging, or OCT. Procedure-related findings noted in vehicle control animals or treated animals included procedure site (injection track) and minimal choroidal mononuclear cell infiltrates. Findings in Group 3 (pH 5.0) were limited to procedure-related findings with no evidence of retinal degeneration/atrophy.

Quantitative ERG analysis from DB showed:

- Mild decrease in the Scotopic -24dB responses in Groups 2 and 3 at Weeks 4 and 8 when compared to baseline values
- Mild decrease in the Scotopic 0dB a-wave responses in Group 2 at Weeks 4 and 8 and in Group 3 at Week 8 when compared to baseline values
- No changes were observed in the Scotopic 0dB b-wave or Photopic Flicker responses

Quantitative ERG analysis from NZW showed no difference in Scotopic or Photopic responses at any time point.



A. Animal 4695 OS: H&E stain 4x, inset 20x
B. Animal 4760 OS: H&E stain 4x, inset 20x
C. Animal 4690 OD: H&E stain 10x, inset 20x
D. Animal 4637 OD: H&E stain 10x

Figure 3. Representative Histologic Findings of Lesions Identified *In Vivo* From Group 2 Animals

Focal retinal degeneration/atrophy was noted in the inferior retina (asterisks) with loss of distinct retinal layers (black arrows) with the lesion margins and unaffected retina at edges of images (white arrows). Findings associated with the foci of retinal degeneration/atrophy included hypertrophy of the retinal pigment epithelium (RPE; (red arrow in Figure A and C insets), vitreal mononuclear infiltrates (red arrowhead in Figure A and C inset) and pigmented cells (macrophages and/or RPE) within the affected retina (black arrowheads in Figure A inset and Figure C) of the DB rabbits.

CONCLUSION

Our results suggest that acidic solutions at pH 4.0 may lead to retinal degeneration, raising concerns about its safety for intravitreal use.

Mild changes in the Scotopic response were observed in groups dosed with acidic solution at pH 4.0 and pH 5.0, suggesting that acidic solutions may interfere with photoreceptor functionality.

Further studies are warranted to assess the long-term effects of acidic solutions in retinal morphology and physiology.

The data presented adds to the knowledge of pH ranges that should be considered for intravitreal drug formulation development.

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