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In Vivo and Histological Analysis of Focal Chorioretinal Defects in Dutch Belted Rabbits

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INTRODUCTION

- Baseline ophthalmic examination including slit lamp and indirect ophthalmoscope is critical in all species involved in ocular toxicity studies.
- Spontaneous ocular lesions identified during prescreening have been reported in mice[1], rats[2], dogs and nonhuman primates[3], involving cornea, iris, lens, vitreous, and retina.
- Laboratory rabbits (Dutch Belted or New Zealand white) are another common species used in ocular toxicology studies, with limited data reporting spontaneous ocular lesions involving cornea, iris, lens and vitreous[4].
- Our purpose is to examine the *in vivo* retinal microanatomy of findings observed during pretest ophthalmic examination in Dutch Belted laboratory rabbits.

METHODS

- Total of six males Dutch Belted rabbits, ranging in age from 7–14 months were used in this study.
- Ophthalmic examinations were performed as baseline prior to ocular studies.
- Retinal changes were documented using a RetCam Shuttle.
- *In vivo* retinal microanatomy was evaluated under general anesthesia by non-invasive imaging with a Confocal scanning laser ophthalmoscopy (cSLO)/Spectral domain optical coherence tomography (sdOCT) instrument (Spectralis® HRA/OCT, Heidelberg Engineering). The cSLO images were taken using the near-infrared (IR), and autofluorescence (AF) modes. The sdOCT images were acquired as raster scans (9 automatic real-time tracking [ART]) or single scan (21 ART).
- At termination eyes were collected and fixed in Davidson's fixative and sent to StageBio or Tissue Vision for histological evaluation.
- At StageBio (Hematoxylin and eosin [H&E] images): 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 H&E.
- At TissueVision (Serial two-photon plus [STP²] Imaging): Eyes were sectioned serially (50 µm thickness) and imaged with a TissueCyte STP² system to visualize tissue AF and collagen content using a 16X Nikon water immersion objective.

Table 1. Ophthalmic Findings and Procedures Performed

Rabbit # (age in months)	Sex	Eye	Ophthalmic Finding	RetCam	OCT	Fluorescence	Histology
1	male	OD	Single round pinkish color ~ 1/2 ONH size superior nasal to optic nerve head	7 months	7 months	Yes	7 months
2	male	OS	Single round pinkish color ~ 1/4 ONH size superior nasal to optic nerve head	7 months	7 months	Yes	7 months
3	male	OD	Single round pinkish color ~ 1/2 ONH size superior temporal to optic nerve head	7 months	7 months	Yes	7 months
4	male	OS	Single round pinkish color ~1/4 ONH size superior temporal to optic nerve head	14 months	14 months	Yes	14 months
5	male	OD	Single ovoid lesion ~ 1/2 ONH size superior temporal	8 months	8 months	No	8 months
6	male	OS	Multifocal roundish lesions <1/4 ONH size superior nasal	6 months	6 months	No	6 months

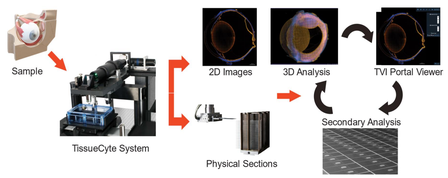

Figure 1. TissueCyte Processing Pipeline

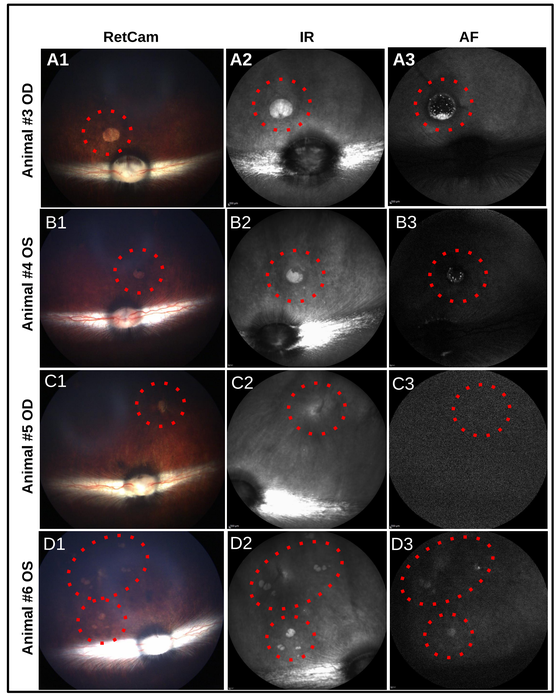

Figure 2. RetCam and cSLO Images

Representative fundus images of four cases presenting retinal abnormalities. **A1, B1**: Fundus color images of round pinkish lesions. **A2, B2**: cSLO images showing lesions as discolored areas in the superior retina that were fluorescent (**A3, B3**) in AF mode. **C1, D1**: Fundus color images of the ovoid and multifocal roundish lesions, respectively. **C2, D2**: cSLO images showing lesions as discolored areas in the superior retina with no fluorescence detected (**C3, D3**) in AF mode. Red circles represent the same area in RetCam, IR and AF images.

RESULTS

- Four rabbits presented unilateral, single, oval-pinkish lesions superior to the optic nerve head. Lesions were autofluorescent under AF mode, and sdOCT showed focal retinal detachment, and autofluorescent material accumulated between photoreceptor and retinal pigment epithelium (RPE) layers. Histological analysis found focal retinal detachment, outer retinal atrophy, and subretinal accumulation of amorphous amphophilic material surrounded by sloughed RPE cells and melanophages.
- One rabbit presented a unilateral, single, oval-light brown lesion at the superior temporal quadrant. This lesion was not autofluorescent under AF mode, and sdOCT showed normal retinal layers, and seemingly focal choroidal atrophy. Histological analysis found a normal retina with a thickened area of choroid extending into the sclera.
- One rabbit presented a unilateral, multifocal roundish lesions at the superior nasal quadrant. These lesions were not autofluorescent under AF mode, and sdOCT showed normal retinal layers. Histological analysis of this eye was not performed.

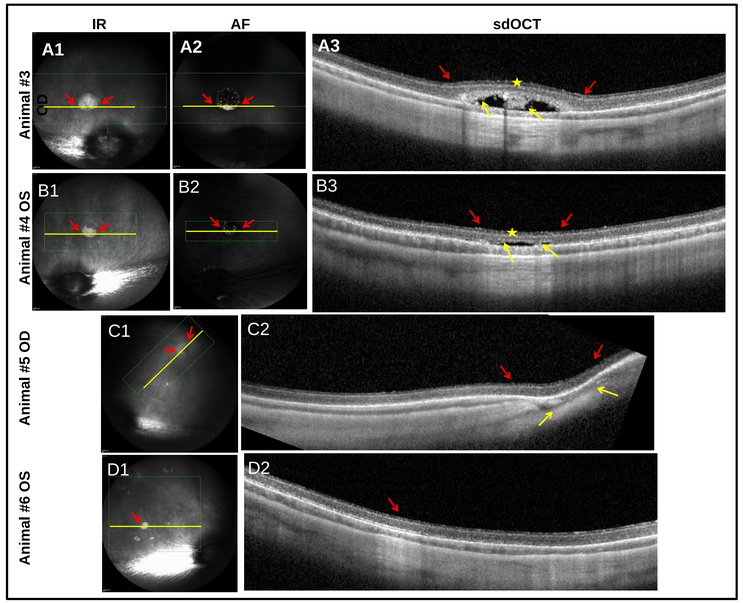

Figure 3. *In Vivo* Images Shows Alterations in Retinal Morphology

cSLO/sdOCT images of lesions observed in this study. **A1, B1**: cSLO images of round pinkish lesions showing a discolored area in the superior retina in near infrared images that were fluorescent (**A2, B2**) in AF mode. **C1, D1**: Near infrared images of the ovoid and multifocal lesions, respectively. No AF images are shown as fluorescence was not present. Yellow horizontal line represents the level of B-scan image shown on the right side. **A3, B3**: IR/sdOCT B-scans of the round pinkish lesions showed a detached retina (yellow asterisks), with some material accumulation in the subretinal space (yellow arrows). **C2**: IR/sdOCT B-scans of the ovoid lesion showed a normal retinal, and seemingly focal choroidal atrophy (yellow arrows). **D2**: IR/sdOCT B-scans of the multifocal roundish lesions showed normal retinal layers and choroid. Red arrows represent same region in IR, AF and sdOCT images.

CONCLUSION

- This is the first report correlating *in vivo* microanatomical and histological description of retinal findings in Dutch Belted rabbits.
- We propose that the AF observed in those cases of single, round pinkish lesions with basal laminar deposits could be due to a deposit of lipofuscin at the RPE level. Further studies are needed to determine the origin of this fluorescence and to characterize the subretinal amphophilic material observed microscopically.
- Baseline ophthalmic examinations in animals enrolled in ocular toxicology studies are imperative in order to identify ocular lesions and determine their inclusion or exclusion in ocular studies.
- We suggest that a different name for changes broadly categorized as chorioretinal scars be considered, as lesions reported do not show fibrotic areas involving the retina or choroid, and either present different anatomical changes in the retina / choroid, or normal retinal structure with only focal pigmentary changes.

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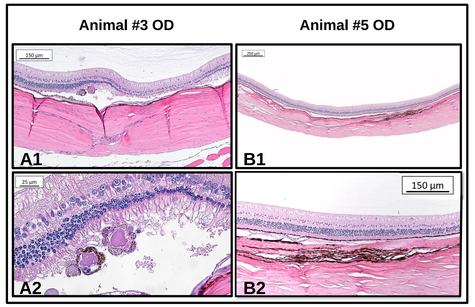

Figure 4. Histological Images

Photomicrographs of the clinically observed lesions noted in Animal #3 and Animal #5. **A1**: Low magnification image showing focal retinal detachment, outer retinal atrophy, and subretinal material accumulation. **A2**: Higher magnification image of A1 showing subretinal accumulation of amorphous amphophilic material surrounded by sloughed RPE cells and melanophages. **B1, B2**: Low and high magnification images showing a histologically normal retina with a thickened area of choroid extending into the sclera.

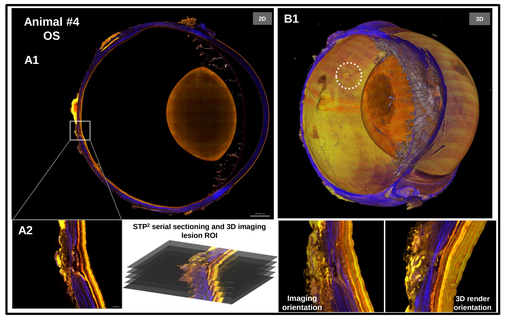

Figure 5. STP² 3D Imaging From Animal #4 Presenting Single Round Pinkish Lesion

A1: 2D section of entire globe depicting lesion location. **A2**: Higher magnification of lesion showing a detached retina and material accumulation in the subretinal space. **B1**: 3D visualization of globe depicting lesion location. Images capturing tissue AF(red/green) and collagen (blue).

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