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Ocular Imaging Followed by Microscopic 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 the cornea, iris, lens, vitreous, and retina.
- Laboratory rabbits (Dutch Belted or New Zealand white) is 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 seven Dutch Belted rabbits (5 males and 2 females), in age from 7–14 months, were used in this study.
- Ophthalmic examinations were performed as a 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 [STP^2] Imaging): Eyes were sectioned serially (50 µm thickness) and imaged with a TissueCyte 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	female	OS	Single round pinkish color <1/4 ONH size superior nasal to optic nerve head	5 months	5 months	Yes	5 months
6	female	OU	Single round pinkish color <1/4 ONH size superior temporal to optic nerve head OD and ~ 1/4 ONH size superior temporal OS	5 months	5 months	Yes	5 months
7	male	OS	Single round pinkish color <1/4 ONH size superior temporal to optic nerve head	6 months	6 months	Yes	6 months

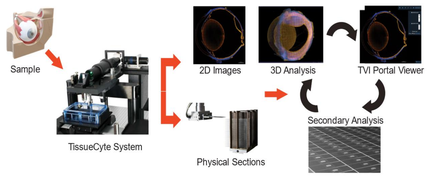

Figure 1. TissueCyte Processing Pipeline

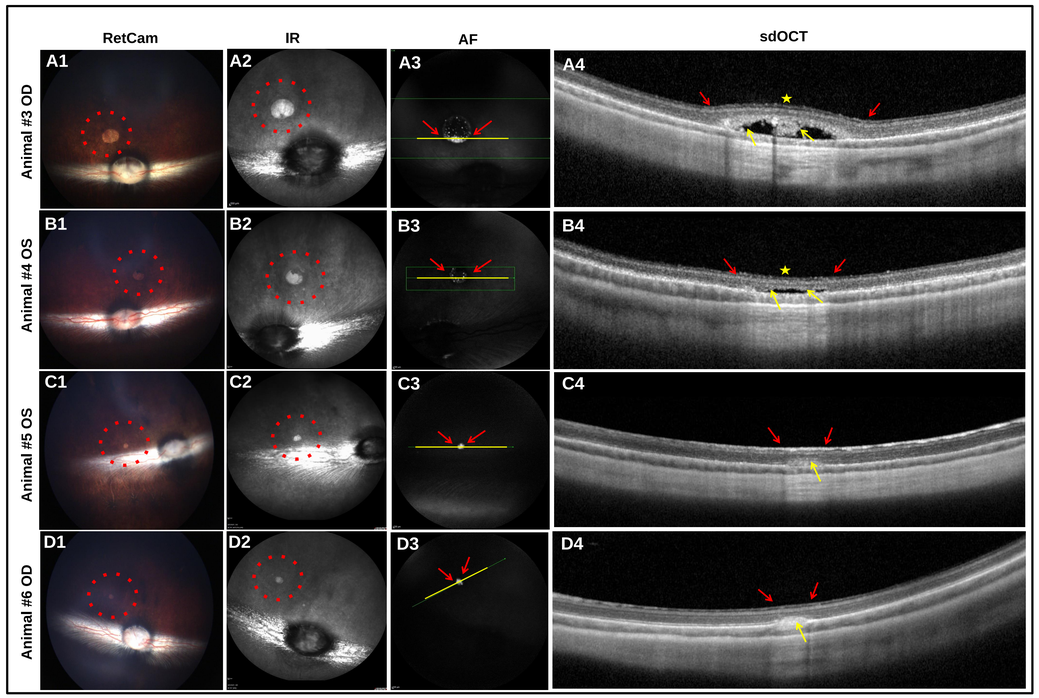

Figure 2. *In Vivo* Images Shows Alterations in Retinal Morphology

Representative fundus images of four cases presenting retinal abnormalities. **A1, B1, C1, D1**: Fundus color images of round pinkish lesions. **A2, B2, C2, D2**: cSLO images showing lesions as discolored areas in the superior retina that were fluorescent (**A3, B3, C3, D3**) in AF mode. The yellow horizontal line represents the level of the B-scan image shown in images A3 - D3. **A4, B4, C4, D4**: IR/sdOCT B-scans showed a detached retina (yellow asterisks) in larger lesions, with some material accumulation in the subretinal space (yellow arrows). Red circles represent the same area in RetCam and IR images, and red arrows represent the same region in AF and sdOCT images.

RESULTS

- Six rabbits presented unilateral and 1 rabbit presented bilateral, single, oval-pinkish lesions superior to the optic nerve head.
- All lesions were autofluorescent under AF mode.
- sdOCT images showed focal retinal detachment in lesions approximately ½ ONH size, and autofluorescent material accumulated between photoreceptor and retinal pigment epithelium (RPE) layers.
- Retinal detachment was not so obvious in lesions ≤ ¼ ONH size.
- Histological analysis found focal retinal detachment, outer retinal atrophy, and subretinal accumulation of amorphous amphophilic material surrounded by sloughed RPE cells and melanophages.

CONCLUSIONS

- This is the first report correlating *in vivo* microanatomical and histological descriptions 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 present different anatomical changes in the retina/choroid.

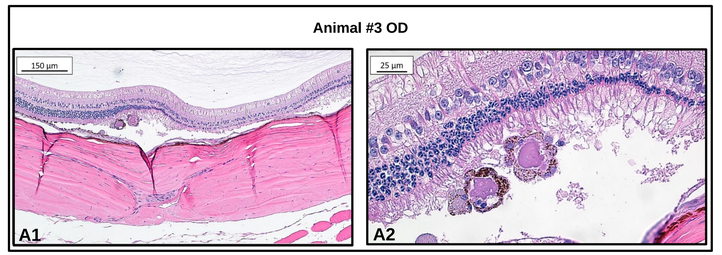

Figure 3. Histological Images

Photomicrographs of the clinically observed lesion noted in Animal #3. **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.

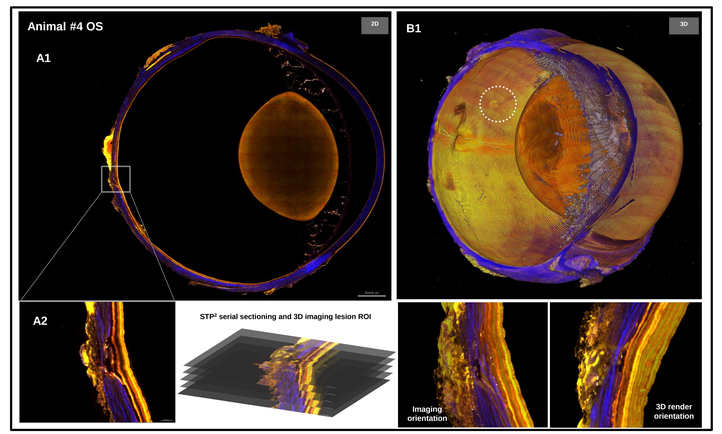

Figure 4. STP^2 3D Imaging

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).

REFERENCES

1. Mukaratirwa S. *Toxicol Pathol* 2015; 43:530-535.
2. Kuno H.J *Vet Med Sci* 1991; 53: 607-614.
3. Shibuya K. *J Toxicol Pathol* 2015; 28: 181-188.
4. Holve DL. *Comp Med* 2011; 61: 436-440.

ACKNOWLEDGEMENTS

- Brandon Ossont, Carrie Krowiak and the staff at Altasciences Preclinical Scranton for animal care support.
- Kristin Mays and the staff at StageBio; Neil Patel and the staff at TissueVision for histology support.
- CR: None.