Ocular heterotopic bone formation in a guinea pig: A case report with 7-month follow-up using advanced ophthalmic imaging technology

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Abstract
Advanced ophthalmic imaging techniques can provide important clinical insights into the pathophysiological changes in heterotopic bone formation (HBF). This case report revealed progression of ocular HBF in the guinea pig over a period of 7 months.

KEYWORDS
cavia porcellus, guinea pig, heterotopic bone formation, ophthalmic examination, optical coherence tomography, osseous metaplasia

1 | INTRODUCTION

Heterotopic bone formation (HBF) or osseous metaplasia/choristoma describes the abnormal growth of bone in muscle and soft tissues.1 Although ocular HBF has been reported across a range of species, it is most often reported in the guinea pig (Cavia porcellus).2,5 with one study, by Williams and Sullivan, reporting a prevalence figure for HBF in the ciliary body of 8 in 1000 guinea pigs.5 Such bony tissue outgrowths appear to originate in the ciliary body, becoming more visible when or if they grow into the iridocorneal angle. Associated discomfort and/or visual problems are generally believed to be unlikely, unless the entire circumference of the iridocorneal angle is affected.

While intraocular osseous metaplasia can develop secondary to penetrating trauma, intraocular infections, and intraocular inflammation, its precise etiology is unknown. However, one hypothesis has linked this condition to the
accumulation of high levels of ascorbic acid (vitamin C) in the aqueous humor, thereby promoting localized mineralization and bone formation in the ciliary body. Interestingly, both guinea pigs and rabbits have been shown to actively concentrate ascorbic acid in the aqueous humor, potentially as a protection against the oxidizing effect of UV radiation. Although deficiencies in vitamin C are known to cause signs of scurvy and other health problems in guinea pigs, over-supplementation with vitamin C is also a plausible risk factor.

Optical coherence tomography (OCT) has become the standard of care in ophthalmology, providing real-time, high-resolution structural information for use in diagnosing ocular disease and helping to understand disease pathogenesis. However, the application of OCT imaging in the evaluation of HBF in guinea pigs has not been reported to-date. The use of this and other ocular imaging technologies has potential to contribute new insights into the progression of HBF in guinea pig, which is not well understood, with previous studies of this condition heavily relying on histological examination of ocular tissue from sacrificed animals.

The primary purpose of this case report is to establish reference ophthalmic findings, which include 7-month follow-up data from one guinea pig diagnosed with HBF.

2 MATERIALS AND METHODS

A 3.8-year-old male guinea pig (1.4 kg; Elm Hill pigmented strain [Elm Hill Labs]) underwent a comprehensive ophthalmic examination after white masses were observed in the peripheral limbal region of the corneas of both eyes by one of the campus veterinarians (co-author KJ), who proposed a tentative diagnosis of HBF. This animal was one of a colony of guinea pigs housed in an approved facility under a 12 hours on/12 h off light cycle, with an average floor luminance of approximately 160-180 lux. These animals have free access to water and are fed a high-fiber guinea pig diet (Teklad 2041, Envigo), along with fresh fruit and vegetables three times a week as dietary enrichment. All animal care and procedures outlined in this report conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experimental protocols were also approved by the Animal Care and Use Committee of the University of California, Berkeley, USA.

The ophthalmic examination included slit lamp biomicroscopy, gonioscopy, anterior segment OCT (AS-OCT; Visante, Carl Zeiss Meditec, Inc), rebound tonometry (iCare; Tonolab), posterior segment spectral domain OCT (SD-OCT; Bioptigen Envisu R-Series), and optical biometry (Lenstar LS900; Haag-Streit AG). Cross-sectional ocular images, including of the HBF with AS-OCT and of the optic nerve head with SD-OCT, were captured for later analysis. From the latter images, overall retinal and nerve fiber layer thicknesses were estimated for each eye, at one location adjacent to the optic nerve head and approximately 700 μm nasally with respect to its center.

All procedures were performed on the animal subject while awake, with the exception of SD-OCT imaging and gonioscopy, for which the subject was anesthetized with a ketamine/xylazine combination (27/0.6 mg/kg body weight), delivered by intraperitoneal injection. One drop of a topical anesthetic (0.5% proparacaine hydrochloride ophthalmic solution, Sandoz Inc) was also instilled prior to gonioscopy, which made use of a custom-designed 5.5-mm four-mirror gonioprism lens (Ocular Instruments, Inc). The eyes of this animal were further evaluated 7 months after the initial evaluation, using the same clinical procedures, that is, slit lamp biomicroscopy, tonometry, AS-OCT, SD-OCT, and optical biometry.

3 RESULTS

Slit lamp biomicroscopy revealed white masses in the anterior chambers of both eyes, adjacent to the limbus both nasally and temporally in the right eye, and superiorly and temporally in the left eye (Figures 1A,B, 2A). High magnification views revealed vascularization of the same (Figure 2A), with cross-sectional and gonioscopic views indicating the location of these masses to be in the space between the cornea and iris (Figure 2B-D). AS-OCT imaging conclusively established these masses to be located between the posterior corneal endothelium and anterior iris surfaces (Figure 3A,B).

Slit lamp biomicroscopy and AS-OCT imaging were repeated 7 months after the initial examination (Figures 1C,D, 3C,D), with comparison of the areal extent of the HBF lesions in these and previously captured images indicating significant progression in both eyes. In the right eye, the masses had extended into both superior and inferior limbal regions (Figure 1A,C) and in the left eye, into nasal region (Figure 1B,D). In terms of the estimated angular extensity of the lesions, values change from 182 to 175 degree for right and left eyes at baseline, to 282 and 278 degree, respectively, 7 months later (Table 1).

Results from tonometry and optical biometry are summarized in Table 1. The IOP of the right eye was initially found to be slightly higher than that of the left eye, by approximately 5 mm Hg, and its axial length was also slightly longer, by 0.11 mm. Seven months later, recorded IOPs were only slightly higher than the earlier readings and still within normal range, although a disparity between right and left eyes was again in evidence, with the right eye's IOP being 5.3 mm Hg higher. The interocular difference in central corneal thickness was only 2 μm and, therefore, unlikely to account for the above IOP differences.
Posterior segment SD-OCT imaging undertaken at the initial examination showed no obvious differences in the appearance of the optic nerve heads of right and left eyes (Figure 4A,B), and both overall retinal and nerve fiber layer thicknesses in the sampled regions, adjacent to the optic nerve head, were similar for the two eyes (Table 1). No detectible changes in either nerve fiber layer thickness or total retinal thickness were observed over the 7 months monitoring period (Table 1).

**FIGURE 1** Heterotopic bone formation of a 3.8- y- old male pigmented guinea pig (Elm Hill strain): Slit-lamp biomicroscopy image of lesion in right eye (A), with white masses evident adjacent to limbus, both nasally (red arrow) and temporally (yellow arrow); image of lesion in left eye (B), with more extensive masses extending superiorly and temporally (blue arrow); seven months follow-up slit-lamp biomicroscopy images of lesions show expansion of and new masses in both eyes; in right eye (C), new masses are evident both superiorly and inferiorly, and in left eye (D), there is a new nasal mass adjacent to limbus.

**FIGURE 2** High magnification slit-lamp biomicroscopy and gonioscopy images of heterotopic bone formation in guinea pig subject: Slit-lamp image of temporal masses in left eye (yellow box in Figure 1B), showing overlying blood vessels (A); cross-sectional slit lamp image confirming location of lesion ie, between but distinct from cornea and iris (B). Superimposed blue arrow indicates corresponding lesion; gonioscopic images of lesions in right eye, in nasal iridocorneal angle (C), and inferior iridocorneal angle (D). Superimposed red arrow indicates corresponding lesion site shown in Figure 1A. AC, anterior chamber.

4 | **DISCUSSION**

Diagnosis of HBF is usually made on clinical appearance alone. While there is no known treatment for the condition, intervention is also typically not considered necessary as affected eyes generally remain healthy and the animals remain comfortable. For the same reasons, histopathologic confirmation of the diagnosis is rarely done.
In the case reported here, the two eyes were differentially affected, in terms of both the location and extensity of the HBF lesions. Nonetheless, there was significant progression in both eyes over a period of 7 months, in terms of both the size and location of the lesions. Both AS-OCT and gonioscopy proved invaluable in evaluating the HBF lesions, which were confirmed to lie in the iridocorneal angle, adjacent to the corneal endothelium rather than within the cornea. The applied clinical techniques together also allowed quantification of the changes over time.

To-date, an association with secondary glaucoma, as a byproduct of accumulation of bone in the iridocorneal angle, has not been definitively established. For example, glaucoma was not recognized clinically in a recent large study of guinea pigs, although a possible association between HBF and glaucoma was raised based on postmortem findings in another study, which unfortunately lacked IOP data. However, while the overall prognosis for HBF appears to be good, given the significant progression observed in just 7 months in the case described here, it would seem prudent to continue with periodic ocular examinations of affected animals to include monitoring of IOP.

The subject of the current study also offers provocative evidence in support of a link between HBF and secondary glaucoma in that the right eye, which showed the most advanced HBF changes, also recorded higher IOPs, by 5 mm Hg, than its fellow at both time points. Moreover, the right eye was also slightly longer than the left eye, raising the possibility of pressure-induced accelerated scleral creep, as also noted in human babies with congenital glaucoma. However, it is important to note that the IOPs of both eyes of this guinea pig subject were still within normal

**TABLE 1** Summary of ocular biometric data collected from a 3.8-y-old male, pigmented guinea pig

|                      | Baseline | 7 mo |
|----------------------|----------|------|
|                      | Right eye | Left eye | Right eye | Left eye |
| Intraocular pressure (mm Hg)(SD) | 18.3 (0.6) | 13.3 (1.2) | 20.3 (0.6) | 15 (1.0) |
| Circumferential extent of lesion (degs) | 182 | 175 | 282 | 278 |
| Axial length (mm)     | 10.40 | 10.29 | 10.99 | 10.86 |
| Cornea thickness (μm) | 249 | 251 | 263 | 252 |
| Mean corneal curvature (D) | 66.48 | 65.36 | 65.69 | 65.97 |
| Astigmatism (D)       | −3.36 | −5.32 | −4.30 | −4.70 |
| Axis                  | 84 | 112 | 112 | 87 |
| Total retinal thicknessa (μm) | 118 | 116 | 119 | 114 |
| Nerve fiber layer thicknessa (μm) | 22 | 22 | 22 | 22 |

Abbreviations: D, diopter; SD, standard deviation.

*aMeasured ~ 700 μm nasal to center of optic nerve head of each eye.*

**FIGURE 3** Anterior segment OCT images of lesions in right (A) and left (B) eyes, at nasal and temporal limbal locations corresponding to intersections of superimposed white lines in Figure 1A,B, respectively. Colored arrows indicate corresponding lesion sites in each set of images (right eye: nasal, red arrow; temporal, yellow arrow; left eye: temporal, blue arrow); equivalent images for right eye (C) and left eye (D), captured at the 7 mo follow-up examination.
limits at the second measurement time point. The latter finding may explain why there were no obvious differences at either time point between the two eyes, in either the gross appearance of their optic nerve heads or the thicknesses of the nearby nerve fiber layer and retina overall, as evaluated by SD-OCT. Nonetheless, the observed increases in the extent of the HBF lesions in both eyes are consistent with it being a progressive condition, and thus, longer-term monitoring may ultimately reveal changes in optic nerve head morphology.

Two different types of ophthalmic OCT imaging instruments were used to examine the eyes of this animal, with each having its own merits. AS-OCT allowed visualization of the lesions, yielding high-quality cross-sectional images of the anterior ocular segment without the need for corneal anesthesia, as required with gonioscopy. However, it should be acknowledged that there are substantial differences in instrument costs, with gonioscopy being more affordable, despite the need for custom-designed gonio lenses, suitable for use on the guinea pig eye. Posterior segment SD-OCT imaging, which was also undertaken in the examination of this subject, offers the possibility of early detection of retinal and optic nerve head structural changes secondary to increases in IOP, as an indirect indicator of visual function loss, when directly testing for the latter is not possible.

In conclusion, ophthalmic imaging techniques have utility in the diagnosis and monitoring of HBF in the guinea pig, allowing tracking of disease progression, with the potential to also offer new insights into its pathophysiology. Longer-term follow-up and additional cases would also be beneficial in this respect.

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CONFLICT OF INTEREST
No financial support or conflicts of interest were identified for this study for all authors.

AUTHOR CONTRIBUTIONS
SG planned and wrote the manuscript. SG, QZ, and JT: together collected the data. KJ and CW: contributed to the interpretation of data, and critically revised the manuscript. All authors approved the final version of the manuscript.

DATA AVAILABILITY STATEMENT
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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