J Toxicol Pathol 2023; 36: 45–48

Case Report

Two cases of cartilaginous metaplasia in the sclera of Japanese White rabbits

Kotaro Yamada[1]*, Yoshinori Yamagiwa[1], Miki Masatsugu[1], and Yu Haranosono[1]

[1] Central Research Laboratories, Senju Pharmaceutical Co., Ltd., 6-4-3 Minatojima-minamimachi, Chuo-ku, Kobe-shi, Hyogo 650-0047, Japan

Abstract: Spontaneous cartilaginous metaplasia of the sclera has not been reported in rabbits. Herein, we report two cases of spontaneous cartilaginous metaplasia in the sclera of Japanese White (JW) rabbits. Case 1 was noted in a 14-week-old male Kbs:JW rabbit that received a single ocular instillation of 20% isoproterenol (IP) a day before necropsy, and showed no abnormalities in clinical signs, ophthalmological assessments, and necropsy. Case 2 was noted in a 38-week-old male Kbs:JW rabbit that was housed under light-emitting diode (LED) lighting for 26 weeks and showed no effects of LED on clinical signs, ophthalmological assessments, and necropsy. Histological sections of the eyes of both animals were prepared and stained with hematoxylin and eosin (H&E) and Alcian blue, and immunohistochemical staining for vimentin was performed. The H&E-stained specimens showed focal hyaline cartilage-like tissues distributed between the scleral fibers at the posterior pole in both cases. The surrounding scleral fibers were compressed and/or partially destroyed by the cartilage-like tissue. The cartilage-like matrix was stained blue by Alcian blue, and immunohistochemistry showed that chondrocyte-like cells were positive for vimentin. Based on these findings, we diagnosed cartilaginous metaplasia in the sclera of Kbs:JW rabbits. The lesion was farther from the IP administration site in Case 1 and was not accompanied by other ophthalmological or histopathological abnormalities in either of the cases. This implies that the lesions occurred spontaneously owing to the abnormal differentiation of neural crest-derived cells. (DOI: 10.1293/tox.2022-0062; J Toxicol Pathol 2023; 36: 45–48)

Key words: clera, eye, cartilage, metaplasia, rabbit

Osseous or cartilaginous metaplasia in the sclera is characterized by focal or multifocal plaques of immature woven bone or cartilage foci. Osseous metaplasia in the sclera is a common alteration in aging Fischer 344 (F344) rats; however, cartilaginous metaplasia is rarely observed[1]. In rabbits, scleral osseous/cartilaginous metaplasia has not been described in the previously published background histopathological data[2], even though their eyes were frequently examined in toxicity studies[3]. Recently, scleral osseous metaplasia was described in the published International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) guide in rabbits[4] and has been reported as a reaction to intraocular implantation of polyethylene polymer implants in New Zealand white rabbits[5]. However, to our knowledge, spontaneous scleral osseous/cartilaginous metaplasia has not been reported in rabbits. We recently encountered two cases of spontaneous cartilaginous metaplasia in the sclera of Japanese White (JW) rabbits. Therefore, this report describes the histopathological characteristics of these cases of cartilaginous metaplasia.

The first case, observed in a male Kbs:JW rabbit, was used to evaluate the effects of ocular instillation of isoproterenol (IP) on the cardiovascular system. The animal received one drop of 20% IP on the ocular surface at 14 weeks of age. No abnormalities were observed in clinical signs, body weight, or slit-lamp biomicroscopy findings before or after the IP administration. A day after the IP administration, the rabbit was euthanized by exsanguination via the abdominal aorta under inhalation anesthesia with sevoflurane, and a necropsy was performed. No abnormalities were observed during the necropsy.

In the second case, the male Kbs:JW rabbit was housed in a room where the light illuminator, fluorescent lamps were replaced by tubular light-emitting diode (LED) bulbs for 26 weeks without any attempt to evaluate the effects of the housing conditions on the eye. The animal showed no effects of LED illumination in terms of clinical signs, body weight, funduscopy, slit-lamp biomicroscopy, or electroretinogram (ERG) results throughout the rearing period. At the end of the rearing period, the rabbit was euthanized by exsanguination via the abdominal aorta under inhalation anesthesia (sevoflurane), and a necropsy was performed at approximately 38 weeks of age. No abnormalities were observed during the necropsy.

Both animals were purchased from Kitayama Labes

Received: 14 June 2022, Accepted: 17 October 2022
Published online in J-STAGE: 2 November 2022
*Corresponding author: K Yamada (e-mail: k-yamada@senju.co.jp)

Co., Ltd. (Nagano, Japan) and individually housed in a stainless steel and plastic cage, R-suite X-Type (Tecniplast S.p.A., Buguggiate, Italy) at a controlled temperature of 22 ± 3°C and relative humidity of 55 ± 15% under a 12-h/12-h light/dark cycle in a conventional animal room. The lighting intensity was set to 300 lx or more and approximately 230 lx in Cases 1 and 2, respectively. Commercial diets, Labo R Stock (Nosan Corporation, Kanagawa, Japan) and LRC4 (Oriental Yeast Co., Ltd., Tokyo, Japan), were provided in Cases 1 and 2, respectively, and *ad libitum* access to tap water was provided. The animal experiments were approved by the Institutional Animal Care and Use Committee of our institution and conducted according to the guidelines for animal welfare of Senju Pharmaceutical Co., Ltd., accredited by AAALAC International.

Both eyeballs and the right eyeball were collected from Cases 1 and 2, respectively, and then fixed with 0.1 M phosphate-buffered 1% formaldehyde/2.5% glutaraldehyde fixative and 10% neutral buffered formalin solution. After fixation, the eyeballs were embedded in paraffin. Sagittal sections of approximately 4-µm thickness were prepared and stained with hematoxylin and eosin (H&E). Additional sections were prepared from the right eyeballs with an abnormal sclera. The sections were stained with Alcian blue solution at pH 2.5 (Fujifilm Wako Pure Chemical Co., Osaka, Japan). Briefly, the sections were deparaffinized with xylene, hydrated in a graded series of ethanol solutions, rinsed, immersed in 3% acetic acid, immersed in Alcian blue solution at pH 2.5, immersed in 3% acetic acid again, rinsed again, counterstained with Kernechtrot (Muto Pure Chemicals Co., Ltd., Tokyo, Japan), rinsed again, dehydrated in a graded series of ethanol solutions, and cleared with xylene. Immunohistochemical analysis was performed using an anti-vimentin mouse monoclonal antibody, V6630 (Sigma-Aldrich, St. Louis, MO, USA), at a dilution of 1:400. Briefly, the sections were deparaffinized with xylene, hydrated in a graded series of ethanol solutions, rinsed, incubated with 3% hydrogen peroxide, rinsed again, immersed in sodium citrate buffer (pH 6.0), heated at 98°C for 40 min, blocked against non-specific protein binding with 10% non-immune goat serum, 50-062Z (Thermo Fisher Scientific Inc., Waltham, MA, USA), incubated with the primary antibody for 60 min, rinsed again, incubated with the secondary antibody, NBP1-75137 (Novus Biologicals LLC, Centennial, CO, USA) for 60 min at a dilution of 1:200, rinsed again, reacted with a diaminobenzidine peroxidase stain DAB kit (Nacalai Tesque Inc., Kyoto, Japan), counterstained with Mayer's hematoxylin solution (Fujifilm Wako Pure Chemical Co., Osaka, Japan), dehydrated in a graded series of ethanol solutions, and cleared with xylene. As a positive control, the trachea of a JW rabbit was treated in the same manner, whereas the primary antibody was omitted in the negative control.

In Case 1, basophilic tissue was focally observed at the posterior pole of the sclera in the right eye after H&E staining. The tissue was distributed between the collagen fiber bundles located in the intermediate layer of the sclera (Fig. 1A). It consisted of a clear pale basophilic cartilage-like matrix and oval or polygonal chondrocyte-like cells within the cavities. The surrounding scleral fibers were compressed and partially destroyed by the cartilage-like tissues (Fig. 1B). The cartilage-like matrix was stained blue with Alcian blue, and it penetrated the scleral fibers (Fig. 1C). Further, immunohistochemical analysis showed that chondrocyte-like cells were vimentin-positive. However, no histopathological findings were observed in the left eye.

In Case 2, a clear pale basophilic tissue was also focally observed at the posterior pole of the sclera after H&E staining. The tissue was distributed in the same area as in Case 1. It consisted of a basophilic cartilage-like matrix and oval cells with foamy cytoplasm. The basophilic matrix penetrated the scleral fibers, and as in Case 1, the cartilage-like tissue somewhat compressed the surrounding scleral fibers (Fig. 2A). The matrix was also stained blue with Alcian blue, as in Case 1 (Fig. 2B), and immunohistochemical analysis showed that chondrocyte-like cells were positive for vimentin.

These lesions were characterized by the appearance of cartilage-like tissues in the sclera, which expanded among the scleral fibers and compressed and/or destroyed the surrounding tissues. Since it has been reported that the sclera in newborn rabbits is histologically immature[6], the histological characteristics we observed above suggest that the cartilage-like tissues were transformed after birth and the formation of the scleral fibers. Alcian blue staining revealed that the cartilage-like matrix contained sulfated acid mucopolysaccharides. In addition, immunohistochemical analysis of vimentin revealed that chondrocyte-like cells were derived from mesenchymal cells as well as scleral cells. Based on these histological features, we diagnosed these lesions as cartilaginous metaplasia in the sclera of Kbs:JW rabbits. We also attempted to detect the expression of collagen II in the cartilage-like matrix with several anti-collagen II antibodies in Case 1, but we could not visualize the antigen because of the lack of a suitable antibody. In both cases, lesions were confined to the posterior pole of the eye. In addition, in Case 1, the location of the lesion was farther from the IP administration site, and no abnormalities were observed in the cornea or adjacent sclera directly exposed to the drug. IP is known to affect the cardiovascular and respiratory systems, metabolism of potassium and glucose, and inflammatory reactions[7]; however, no study has reported the occurrence of scleral cartilaginous metaplasia caused by systemic administration of IP. In Case 2, the scleral cartilage appearance was not accompanied by ERG, fundus, or other histological changes, such as inflammation or trauma, throughout the rearing period under LED lighting. Therefore, the lesions were considered to have occurred spontaneously and were not caused by IP administration or rearing under LED lighting. Although the location of the lesion may be one of the characteristics of spontaneous scleral metaplasia in rabbits, we were unable to calculate the rate of incidence of lesions and strain differences because of the limited number of cases we had access to. Further investigations regarding this

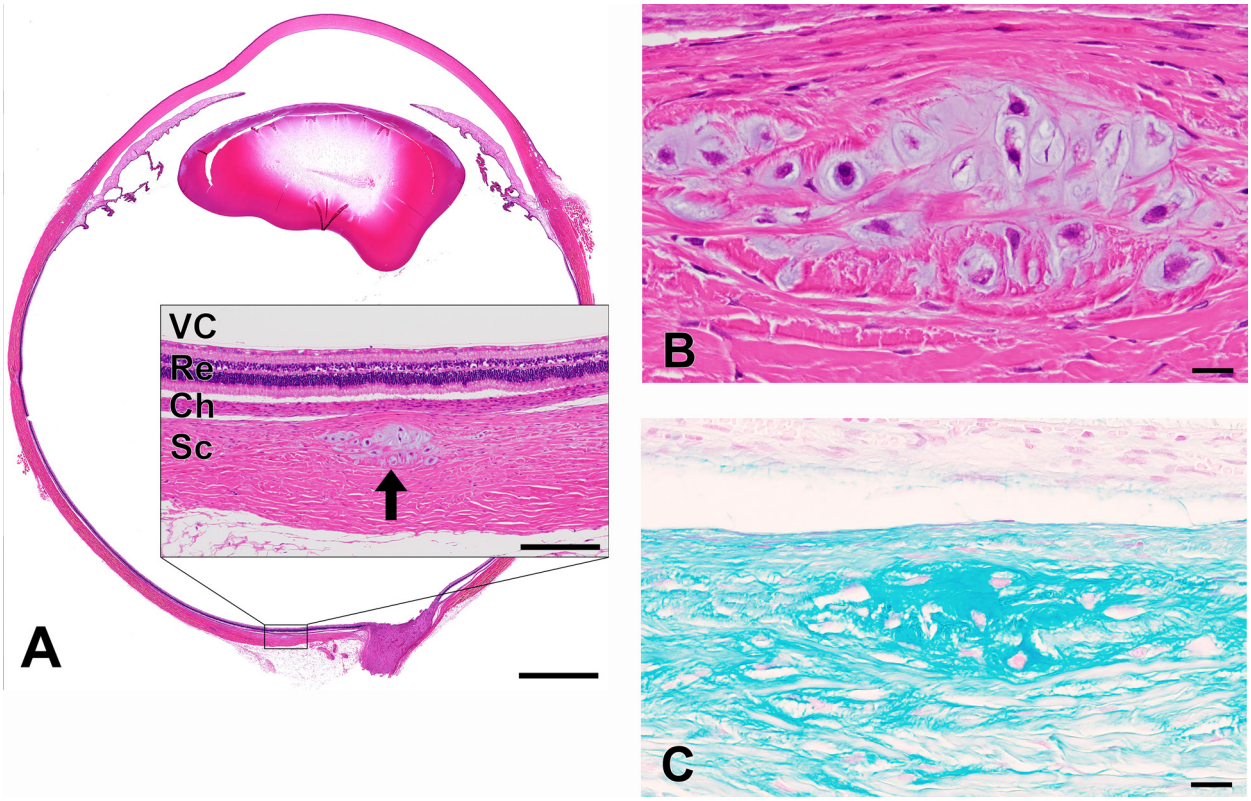

Fig. 1. Right eye of a 14-week-old male Japanese White rabbit.
A: A lesion (arrow) is focally observed at the posterior pole of the sclera (H&E, bar=2 mm). *Inset:* basophilic tissue is focally observed at the posterior pole of the sclera (H&E, bar=200 μm). VC: vitreous cavity; Re: retina; Ch: choroid; Sc: sclera.
B: High magnification of A. The tissue is observed between the collagen fiber bundles located in the intermediate layer of the sclera. The lesion consists of a basophilic cartilage-like matrix and oval or polygonal chondrocyte-like cells within the cavities. The surrounding scleral fibers are compressed and partially destroyed by the cartilage-like tissue (H&E, bar=20 μm).
C: The cartilage-like matrix is stained in blue and penetrates the scleral fibers (Alcian blue staining, bar=20 μm).

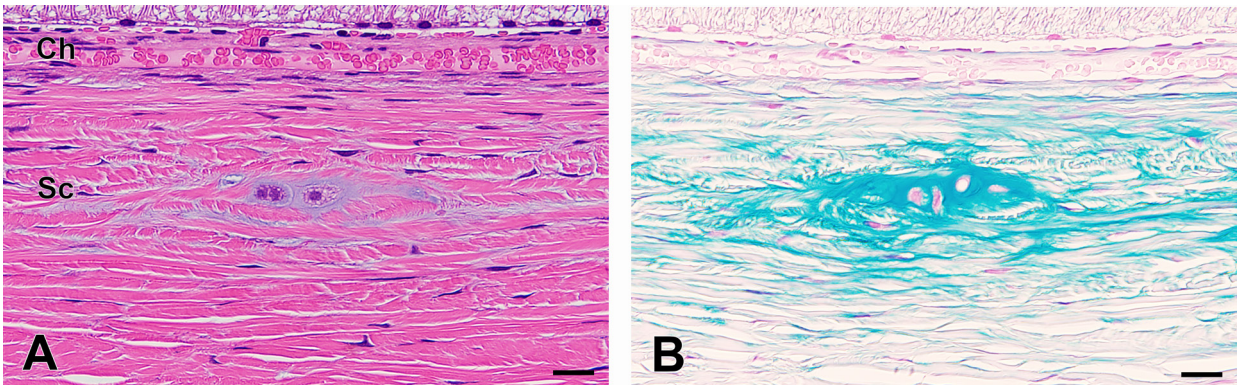

Fig. 2. Right eye of a 38-week-old male Japanese White rabbit. A lesion is focally observed at the posterior pole of the sclera.
A: The tissue consists of a basophilic cartilage-like matrix and oval cells with foamy cytoplasm. The basophilic matrix penetrates the scleral fibers, and as in Case 1, the cartilage tissue compresses the surrounding scleral fibers (H&E, bar=20 μm). Ch: choroid; Sc: sclera.
B: The cartilage-like matrix is stained in blue (Alcian blue staining, bar=20 μm).

issue and studies of different rabbit strains are needed.

Many vertebrates possess scleral bones/cartilage. Teleost fish, birds, lizards, and turtles have both scleral bones and cartilage. Several amphibians have scleral cartilage during the tadpole stages, which is then resorbed during metamorphosis[8]. In contrast, almost all mammals normally lack

scleral bone and cartilage. However, scleral bone or cartilage has been reported to form abnormally in some species. In the F344 rat strain, osseous metaplasia of the sclera occurs in nearly half and more than 80% of aging females and males, respectively[9]. In humans, senile scleral plaques (SSP), a degenerative condition manifesting clinically as an area of grayish discoloration on the sclera and occasionally containing a bone-like matrix, have been reported[10, 11]. It is worthy of note that scleral osseous metaplasia in rodents histologically resembles the SSP in humans[8]. In addition, cartilage and/or bone formation have been reported in approximately half of the examined eyes of domestic goats[12]. Likewise, in sheep, scleral cartilaginous metaplasia has also been reported to occur[13]. Bone and cartilage formation within the sclera is generally caused by aging or secondary changes associated with injury[1]. In humans, the relationships between bone/cartilage formation and several symptoms or treatments have been discussed, including dehydration, mechanical stresses, solar irradiation, gout, rheumatoid arthritis, porphyria, genetic factors, and sequelae of cataract surgery; however, the cause of the appearance of SSP has not been identified[10]. In these cases, the animals showed no evidence of scleral trauma, inflammation, or other abnormalities except for scleral cartilage formation. Scleral cells originate from neural crest-derived cells as well as chondrocytes, and the sclera in humans maintains chondrogenic potential[14]. Thus, the sclera could also maintain chondrogenic potential in rabbits, even in young individuals, suggesting that the cause of the lesions may be the abnormal differentiation of neural crest-derived cells. To the best of our knowledge, this is the first report describing spontaneous cartilaginous metaplasia in the sclera of rabbits.

Disclosure of Potential Conflicts of Interest: The authors declare no potential conflicts of interest regarding the research, authorship, or publication of this article.

Acknowledgments: We thank Heijiro Onishi (Senju Pharmaceutical Co., Ltd.) for providing information on the treatment, examination results, and housing conditions of Case 2.

References

1. Ramos MF, Baker J, Atzpodien EA, Bach U, Brassard J, Cartwright J, Farman C, Fishman C, Jacobsen M, Junker-Walker U, Kuper F, Moreno MCR, Rittinghausen S, Schafer K, Tanaka K, Teixeira L, Yoshizawa K, and Zhang H. Nonproliferative and proliferative lesions of the rat and mouse special sense organs (ocular [eye and glands], olfactory and otic). J Toxicol Pathol. **31**(Suppl): 97S–214S. 2018. [Medline] [CrossRef]
2. Langevin NE, Schafer KA, Turner OC, McPherson BJ, and Rose RE. Historical data: Histopathology lesions observed in the eyes of control rabbits in topical ocular administration and contact lens studies. Toxicol Pathol. **46**: 799–820. 2018. [Medline] [CrossRef]
3. Kurata M, Yamagiwa Y, Haranosono Y, and Sakaki H. Repeated-dose ocular instillation toxicity study: a survey of its study design on the basis of common technical documents in Japan. Fundam Toxicol Sci. **4**: 95–99. 2017. [CrossRef]
4. Bradley AE, Wancket LM, Rinke M, Gruebbel MM, Saladino BH, Schafer K, Katsuta O, Garcia B, Chanut F, Hughes K, Nelson K, Himmel L, McInnes E, Schucker A, and Uchida K. International harmonization of nomenclature and diagnostic criteria (INHAND): Nonproliferative and proliferative lesions of the rabbit. J Toxicol Pathol. **34**(Suppl): 183S–292S. 2021. [Medline] [CrossRef]
5. Fernandez-Bueno I, Di Lauro S, Alvarez I, Lopez JC, Garcia-Gutierrez MT, Fernandez I, Larra E, and Pastor JC. Safety and biocompatibility of a new high-density polyethylene-based spherical integrated porous orbital implant: an experimental study in rabbits. J Ophthalmol. **2015**: 904096. 2015. [Medline] [CrossRef]
6. Yamagiwa Y, Kurata M, and Satoh H. Histological features of postnatal development of the eye in white rabbits. Toxicol Pathol. **49**: 419–437. 2021. [Medline] [CrossRef]
7. Sears MR. Adverse effects of beta-agonists. J Allergy Clin Immunol. **110**(Suppl): S322–S328. 2002. [Medline] [CrossRef]
8. Franz-Odendaal TA. Skeletons of the eye: an evolutionary and developmental perspective. Anat Rec (Hoboken). **303**: 100–109. 2020. [Medline] [CrossRef]
9. Yoshitomi K, and Boorman GA. Eye and associated glands. In: Pathology of the Fischer Rat—Reference and Atlas. GA Boorman, SL Eustis, MR Elwell, CA Montgomery Jr, WF MacKenzie (eds). Academic Press, San Diego New York London. 239–260. 1990.
10. Moseley I. Spots before the eyes: a prevalence and clinico-radiological study of senile scleral plaques. Clin Radiol. **55**: 198–206. 2000. [Medline] [CrossRef]
11. Manschot WA. Senile scleral plaques and senile scleromalacia. Br J Ophthalmol. **62**: 376–380. 1978. [Medline] [CrossRef]
12. Tusler CA, Good KL, Maggs DJ, Zwingenberger AL, and Reilly CM. Gross, histologic, and computed tomographic characterization of nonpathological intrascleral cartilage and bone in the domestic goat (Capra aegagrus hircus). Vet Ophthalmol. **20**: 214–221. 2017. [Medline] [CrossRef]
13. Smith JD, Hamir AN, and Greenlee JJ. Cartilaginous metaplasia in the sclera of Suffolk sheep. Vet Pathol. **48**: 827–829. 2011. [Medline] [CrossRef]
14. Seko Y, Azuma N, Takahashi Y, Makino H, Morito T, Muneta T, Matsumoto K, Saito H, Sekiya I, and Umezawa A. Human sclera maintains common characteristics with cartilage throughout evolution. PLoS One. **3**: e3709. 2008. [Medline] [CrossRef]