Case Report

Glaucoma in a New Zealand White Rabbit Fed High-cholesterol Diet

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Abstract: Goniodysgenesis, malformation of the filtration angle, was observed in a New Zealand white rabbit supplied with 100 g/day rabbit chow containing 0.2% cholesterol for 10 months. Histopathology revealed cupping of the optic disc, atrophy of the retina and hyalinization of the ciliary body in the bilateral eyeballs. These findings corresponded with histopathological features caused by glaucoma. On the basis of these findings, we diagnosed this lesion as glaucoma, and classified it as primary glaucoma because of the presence of developmental defects of the filtration angle. In this case, hypercholesterolemia-induced changes, such as aggregation of lipid-laden macrophages and cholesterol clefts in the sclera or choroid, might cause deterioration of the lesions in glaucoma. (DOI: 10.1293/tox.25.51; *J Toxicol Pathol* 2012; 25: 51–53)

Key words: New Zealand white rabbit, glaucoma, spontaneous

Glaucoma is a pathophysiological state characterized by a prolonged increase in intraocular pressure; it occurs commonly in dogs, less commonly in cats and horses and rarely in other species¹. In rabbits, it is known that inherited glaucoma occurs in the New Zealand white rabbit and other breeds^{2,3}. In this report, we describe the histological characteristics of spontaneous glaucoma in a rabbit fed a high-cholesterol diet.

All experimental procedures were conducted after approval of the study by the Institutional Animal Care and Use Committee of Shionogi Research Laboratories. The experiment was conducted to develop an animal model for arteriosclerosis. Fifty 12-week-old male rabbits were purchased from Kitayama Labes (Nagano, Japan). The rabbits were individually housed in stainless steel cages, maintained under nonbarrier conditions, and supplied with 100 g/day rabbit chow containing 0.2% cholesterol (Oriental Kobo, Osaka, Japan) for 10 months. When the experimental period was over, many white nodules in the iris or opacity in the cornea were macroscopically observed. Five rabbits that exhibited macroscopic changes in the eyeballs were examined histopathologically. Eyeballs were fixed in 10% neutral-buffered formalin and then embedded in paraffin. Paraffin sections were stained with hematoxylin and eosin (H&E) for histopathological examination. Additionally, frozen sections were

subjected to Oil Red O staining and examined. For immunohistochemical examination, the sections were examined with anti-rabbit macrophage antibody (DakoCytomation, Kyoto, Japan). Endogenous peroxidase was inactivated using 3% H₂O₂ and nonspecific proteins were blocked with normal goat serum. Sections were then incubated with the primary antibody overnight at 4 °C at a dilution of 1/200. Immunolocalization was performed using the avidin-biotin peroxidase complex method (DAKO) with 3,3'-diaminobenzidine as the chromogen and counterstaining with hematoxylin. Histopathologically, goniodysgenesis, a continuation of iris-like mesenchyme across the trabecular meshwork inserting into the termination of Descemet's membrane, was observed in the filtration angle in one of the five animals (Fig. 1a). This change was observed bilaterally. In the eyeballs of this animal, there were also retinal atrophy, which was prominent in the inner nuclear layer and ganglion cells (Fig. 2a), cupping of the optic disk (Fig. 3) and hyalinization of the ciliary body (Fig. 4a)¹. These findings were not observed in the other 4 animals (Fig. 1b, 2b and 4b). On the basis of these results, this case was diagnosed as glaucoma because the lesions observed in the animal were known to be caused by glaucoma.

Glaucoma is divided into two types: primary glaucoma and secondary glaucoma¹. Primary glaucoma refers to cases without evidence of prior ocular disease and, in practical terms, is synonymous with malformation of the filtration angle. Secondary glaucoma is caused by antecedent ocular disease, such as intraocular neoplasia and anterior uveal inflammation with posterior or anterior synechiae. This case can be classified as primary glaucoma because of the finding of goniodysgenesis, that is developmental defects of the filtration angle. In the New Zealand white rabbit, hereditary glaucoma has been reported, and histopathological features observed in the present study were similar to those reported previously^{3,6}.

Besides the findings related to glaucoma, there were

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Fig. 1.

Fig. 2.

Fig. 3.

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Fig. 6.

findings that were considered to be caused by hypercholesterolemia^{4,5}, such as aggregation of foamy cells and/or cholesterol clefts observed in the choroid (Fig. 2), sclera, iris, ciliary body and cornea. These changes were mostly observed around the blood vessels. These foamy cells were positive for anti-rabbit macrophage antibody (Fig. 5) and Oil Red O staining (Fig. 6). From these results, it was confirmed that the foamy cells were lipid-laden macrophages. These findings were also observed in the 4 other rabbits, which showed white nodules in the iris or opacity in the cornea. Since accumulation of lipid-laden macrophages around the route of aqueous humor or formation of a mass might cause secondary glaucoma^{7,8}, there remained a possibility of secondary glaucoma. However, in this study, there were few macrophages around the filtration angle (Fig. 1a) and no solid mass formation, and the findings of glaucoma were not observed in other animals. Taking the findings together, the glaucoma observed in the one rabbit is considered to be primary not secondary. However, there are two routes of elimination of aqueous humor: trabecular outflow and uveoscleral outflow¹. In this case, many macrophages and cholesterol clefts were accumulated in the choroid and sclera, especially around the vessels, so there is a possibility that hypercholesterolemia-induced lesions might cause deterioration of the glaucoma by preventing the elimination of aqueous humor.

Primary glaucoma caused by maldevelopment of the filtration angle is frequently encountered in dogs. This has been predominantly described in the American cocker spaniel, Bouvier des Flandres, Siberian husky, and flat-coated retriever among others^{9,10}. For example, defect of the filtration angle was observed in 34% of flat-coated retrievers¹⁰. It has been reported that inherited glaucoma occurs in the rabbit and that it is a suitable animal model of goniodysgenetic glaucoma in humans². In terms of secondary glaucoma, there have been some reports of experimentally induced glaucoma. Rabbits with glaucoma induced by α -chymotrypsin have been widely used to study the long-term effects of elevated intraocular pressure^{11,12}. The pathogenesis of α -chymotrypsin glaucoma seems to be associated with blockage of the filtration angle by the lysed material, inflammation, lens dislocation and vitreous disturbance. These findings are clearly different from those of spontaneous glaucoma.

In this report, we described the histological features of spontaneous glaucoma in a rabbit fed a high-cholesterol

diet, in which the lesions caused by hypercholesterolemia might cause deterioration of the glaucoma.

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Fig. 1. Histopathological findings for the filtration angle. Goniodysgenesis, a continuation of iris-like mesenchyme across the trabecular meshwork inserting into the termination of Descemet's membrane, is observed in one of the five animals supplied cholesterol-containing chow (b). This change is not observed in the other animals (a). Arrows indicate the filtration angle. H&E stain. Bar=100 μ m.

Fig. 2. Histopathological findings in the retina. Retinal atrophy is prominent in the inner nuclear layer and ganglion cells (b). This change is not observed in the other animals (a). Aggregation of foamy macrophages and cholesterol clefts are also observed in the choroid. H&E stain. Bar=100 μ m.

Fig. 3. Histopathological findings in the optic papilla. Cupping of optic disk is observed. H&E stain. Bar=200 μ m.

Fig. 4. Histopathological finding in the ciliary body. Hyalinization of the ciliary body is observed (b). This change is not observed in the other animals (a). Arrows indicate the ciliary body. H&E stain. Bar=100 μ m.

Fig. 5. Immunohistochemistry for anti-rabbit macrophages in the iris. The foamy cells are positive for anti-rabbit macrophage antibody (arrow). Immunohistochemical staining, counterstained with hematoxylin. Bar=100 μ m.

Fig. 6. Oil Red O staining in the sclera. The foamy cells are positive for Oil Red O staining (arrow). Bar=100 μ m.