



NOTE

Pathology

Vascular smooth muscle lipofuscinosis occurring predominantly in veins of a cynomolgus monkey (*Macaca fascicularis*)

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ABSTRACT. A 6-year-old male cynomolgus monkey showed chronic wasting. No gross abnormalities were observed in necropsy except for changes secondary to wasting. Microscopic examination revealed pigment granules deposition in systemic smooth muscles. They were observed as brown or basophilic in hematoxylin and eosin stain, and were positive for periodic acid-Schiff, Schmorl and Ziehl-Neelsen. Ultrastructurally, they consisted of residual bodies surrounded with varying amounts of solitary ribosomes. Thus, these granules were considered as lipofuscin. Unlike brown bowel syndrome in humans, the pigment granules were distributed systemically not only in the digestive tract but also in the blood vessels predominantly in the veins. To our knowledge, this is the first report on vascular smooth muscle lipofuscinosis occurring predominantly in the veins of primates.

KEY WORDS: cynomolgus monkey, lipofuscinosis, smooth muscle, vein

J. Vet. Med. Sci.

83(3): 469–472, 2021

doi: 10.1292/jvms.20-0676

Received: 29 November 2020

Accepted: 6 January 2021

Advanced Epub:

26 January 2021

Lipofuscin is a yellowish brown, electron-dense inclusion that accumulates in cells with age or from various pathological conditions. Lipofuscin is an intralysosomal pigment formed by the peroxidation of unsaturated fatty acids derived from membranes of organelles. It occurs in a variety of tissues and cell types, such as neurons, myocytes, hepatocytes, skeletal muscle and smooth muscle [6, 10]. Lipofuscin deposition in smooth muscle are reported in some cases, such as brown bowel syndrome in humans, brown gut in dogs, and ceroid-lipofuscinosis, an inherited lysosomal storage disease, in several animal species [2, 3, 5, 7–9, 11]. To our knowledge, however, there has been no report describing vascular smooth muscle lipofuscinosis occurring predominantly in the veins of primates including humans. We encountered a 6-year-old cynomolgus monkey (*Macaca fascicularis*) with vascular smooth muscle lipofuscinosis occurring predominantly in the veins. This report describes the histopathological and ultrastructural characteristics of the case and discusses the possible pathogenesis.

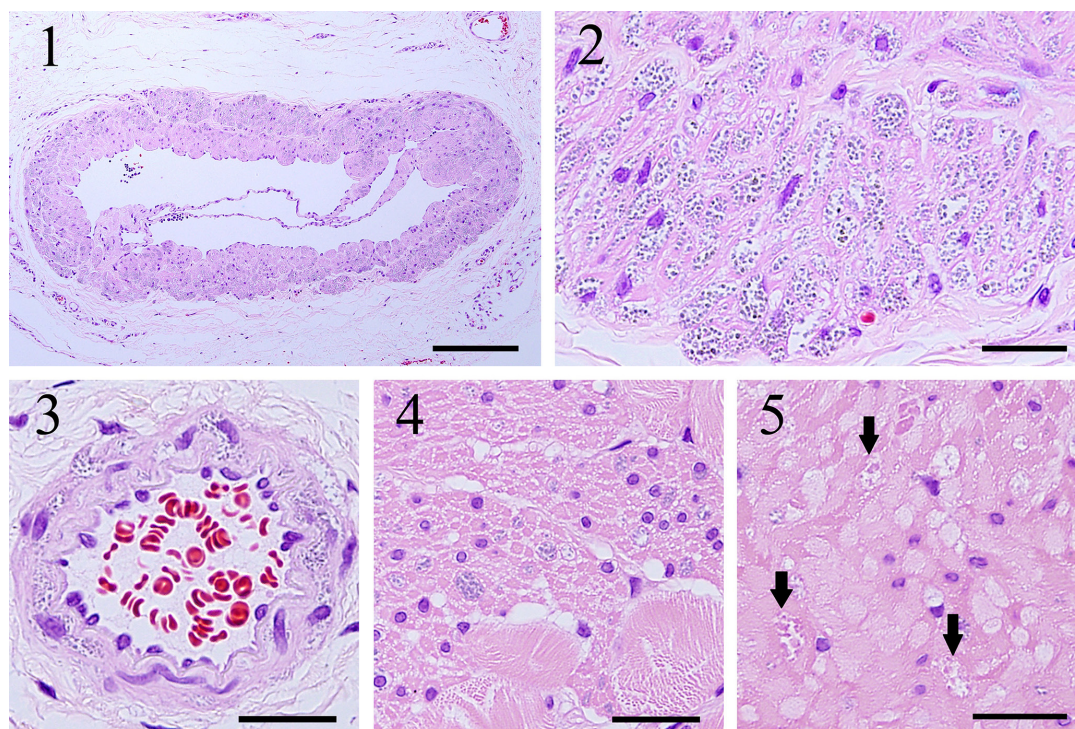
The monkey was imported from Vietnam (purchased from Nafovanny, Vietnam) at 4 years and 2 months of age and was subjected to a background data collection study. It was housed in a metal cage (680 × 608 × 770 mm) in a conventional room air-conditioned at 23°C to 29°C with 35% to 75% relative humidity and a 12-hr light/12-hr dark cycle. It was provided with 100 g of commercially available food (CMK-2; CLEA Japan, Inc., Tokyo, Japan) daily and was allowed free access to drinking water. It was cared for according to the principles outlined in the guides for the care and use of laboratory animals prepared by the Japanese Association for Laboratory Animal Science and our institution. This animal had received no prior treatment. The body weight decreased over 2 years from the time it was imported (3.9 kg) to necropsy (2.4 kg), but the food consumption did not decrease during housing. Diarrhea and vomiting of food residue were observed a month prior to necropsy. Finally, this animal was euthanized by intravenous injection of sodium pentobarbital due to deteriorating general condition at 6 years old. At necropsy, no gross abnormalities were observed except for small thymus and gelatinous atrophy in femoral bone marrow, as changes secondary to wasting. Samples from a range of organs, heart, aorta, vena cava, lung, trachea, kidney, urinary bladder, salivary gland, liver, gallbladder, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, thyroid/parathyroid, adrenal, spleen, thymus, mesenteric lymph node, mandibular lymph node, testis, epididymis, seminal vesicle, prostate, brain, spinal cord, pituitary, eye, optic nerve, lacrimal gland, femoral/sternal bone/bone marrow, femoral muscle, sciatic nerve and skin, were fixed in 10% phosphate buffered formalin (10% PBF) and were embedded in paraffin and cut at 4 μm. Sections were stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), Schmorl, Ziehl-Neelsen, Berlin blue, Kossa and eosin only stains. Histopathological examination revealed brown or basophilic granule deposition in systemic smooth muscles in HE

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- Fig. 1.** Brown or basophilic granules are remarkably observed in the smooth muscles of the vein in the adventitia of the esophagus. Hematoxylin and eosin. Bar, 200 μ m.
- Fig. 2.** Various-sized brown or basophilic pigment granules are distributed diffusely within the cytoplasm or observed around the nuclei of the smooth muscles. Higher magnification of the tunica media shown in Fig. 1. Hematoxylin and eosin. Bar, 25 μ m.
- Fig. 3.** Pigment granules are observed in the smooth muscles of the artery in the adventitia of the esophagus. Hematoxylin and eosin. Bar, 25 μ m.
- Fig. 4.** Pigment granules are observed in the smooth muscles of the muscle layer in the esophagus. They are not observed in the striated muscles of the esophagus. Hematoxylin and eosin. Bar, 25 μ m.
- Fig. 5.** Rarely, eosinophilic granules are observed (arrows) in the smooth muscles of the duodenum. Hematoxylin and eosin. Bar, 25 μ m.

stain. Prominent deposition of pigment granules was noted in the veins of systemic organs, from muscular venules to vena cava (Figs. 1 and 2). The same pigment granules were also observed in the arteries, from arterioles to medium-sized muscular arteries (Fig. 3), and smooth muscle layer of the digestive tract (Fig. 4). The distribution and severity of pigment granules deposited in the smooth muscle of the systemic organs are listed in Table 1. In neurons of the brain and spinal cord, myocytes and hepatocytes, no pigment granules were observed. In the smooth muscles, various-sized pigment granules were distributed diffusely within the cytoplasm or observed around the nuclei. No degenerative or necrotic changes were found in the smooth muscles. The pigment granules stained positive for PAS, dark green with Schmorl, dark red with Ziehl Neelsen, and were negative for berlin blue and Kossa (Fig. 6a–e). In eosin only stain, pigment granules were yellowish brown (Fig. 6f). These suggests that pigment granules were originally yellowish-brown and modified by a basophilic substance. Rarely, eosinophilic granules are observed in the smooth muscles of the blood vessels and digestive tract in HE stain (Fig. 5); however, these granules did not have the same staining characteristics in special stains as the brown or basophilic granules described above. In some areas of the small intestine, mild atrophy of the villi and inflammatory cell infiltration were observed. For transmission electron microscopy, the specimens fixed in 10% PBF were cut from the vein in the adventitia of the esophagus and the outer smooth muscle layer of the rectum. They were washed in water for 12 hr and cut into small blocks. They were post-fixed in 1% osmium tetra-oxide and embedded in epoxy resin. Ultra-thin sections were mounted on copper grids, stained with uranyl acetate and lead citrate, and examined with an H-7600 transmission electron microscope (Hitachi High-Tech Fielding Co., Tokyo, Japan). Ultrastructurally, the pigment granules were consistent with round or irregularly shaped residual bodies with one or more lucent lipid droplets. They were distributed diffusely within the cytoplasm or confined around the nuclei. They were accompanied with various amounts of remnants of organelles and/or myelinoid membranes. Also, various amounts of solitary ribosomes accumulated in the background of cytoplasm. Together with the results of histopathological nature of the pigment granules, it was judged to be lipofuscin. In detailed observation, the cluster of residual bodies was categorized into three types: immature residual bodies with few ribosomes, immature or mature residual bodies with mild to moderate amounts of ribosomes, and mature residual bodies with numerous ribosomes (Fig. 7a–c).

Histopathological examination revealed eosinophilic, brown or basophilic granules deposition in smooth muscles in HE stain. Ultrastructurally, the granules consisted of three types of clusters of residual bodies, and the amount of solitary ribosomes increased

Table 1. Distribution and severity of the pigment granules deposition

	Vein	Artery	Muscle layer
Esophagus	+++	++	++
Stomach	++	+/-	+
Duodenum	+/-	-	++
Jejunum	+	+	+
Ileum	++	-	++
Cecum	+	-	-
Colon	+/-	-	+
Rectum	+	+/-	++
Urinary bladder	-	-	++
Kidney	+++	+	
Lung	++	-	
Pancreas	+++	++	
Thymus	+++	-	
Epididymis	++	-	
Mesenteric lymph node	+++	++	
Mandibular lymph node	++	-	

-, not remarkable; +/-, minimal; +, mild; ++, moderate; +++, severe.

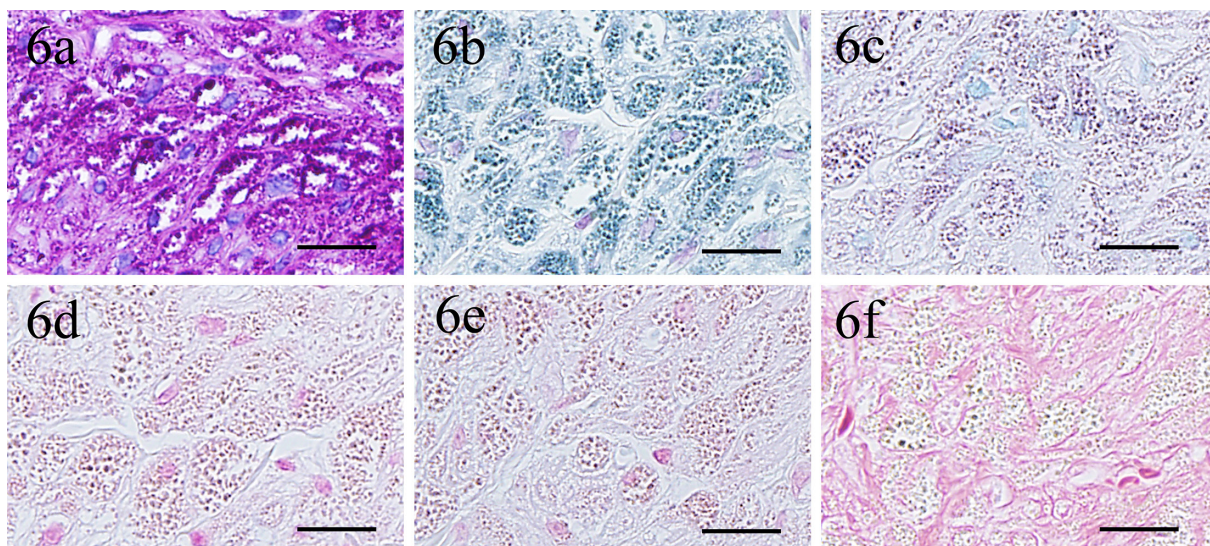


Fig. 6. Special stains of the pigment granules in the tunica media of the veins of the esophagus. (a), Periodic acid-Schiff (b), Schmorl (c), Ziehl-Neelsen (d), Berlin blue (e), Kossa (f), eosin only stain. Bars, 20 μ m.

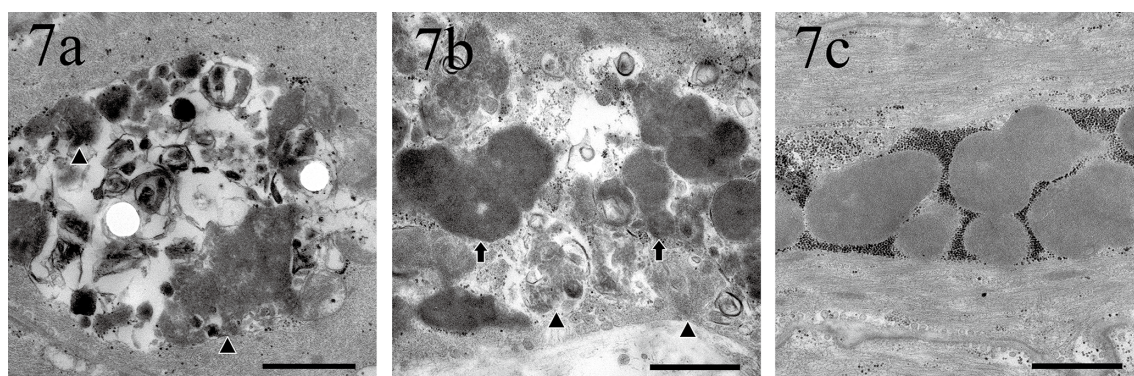


Fig. 7. The cluster of the residual bodies are categorized into three types (a-c). The residual bodies are uniformly round (c), irregularly-shaped (b, arrows) or only dense-granular materials without membrane (a, b, arrowheads). The amount of solitary ribosomes are few (a), moderate (b) or numerous (c). Electron micrograph. Bars, 1 μ m.

as the residual bodies matured. Therefore, it was assumed that basophilic characteristics of the lipofuscin reflected the increase in solitary ribosomes. The rare eosinophilic granules were assumed to be consistent with the most immature ones, indicating an earlier stage of lipofuscin formation.

It has not been reported that lipofuscin stains basophilic as a result of accumulation of solitary ribosomes. Accumulation of solitary ribosomes are known to be observed in disaggregation of polyribosomes which is associated with depression or arrest of protein synthesis [4]. In the liver of carbon-tetrachloride-poisoned rats, vesiculation of rough endoplasmic reticulum and numerous solitary ribosomes are observed in hepatocytes. It has been shown that after carbon tetrachloride poisoning, the synthesis of several export proteins becomes depressed, and cell fractionation studies show that the defect is related to polyribosome disaggregation and a decreased capacity to incorporate amino acid [4]. It is possible that any events associated with depression or arrest of protein synthesis occurred in the present case.

In non-human primates, a few case of lipofuscinosis has been reported, however they are neuronal ceroid-lipofuscinosis mainly in central nervous system [1, 5]. Deposition in the smooth muscles has been reported as part of systemic lipofuscinosis in a non-human primate and other species [5, 7, 8]. In human, smooth muscle lipofuscinosis has been reported as brown bowel syndrome, a rare condition characterized by deposition in the intestinal smooth muscles. In a fraction of patients with brown bowel syndrome, lipofuscin deposition in the smooth muscles of the blood vessels has been reported [9]. However in most cases of brown bowel syndrome, lipofuscin deposited predominantly in the muscle layer of the digestive tract [2, 9]. Thus, the present case is different from these previously reported cases, and it was assumed that there was some predisposition to lipofuscin deposition specifically in the vascular smooth muscles.

In the present case, the pathogenesis of lipofuscin deposition and its relationship to the clinical manifestation remain unclear. In humans, brown bowel syndrome occurs in association with malabsorption syndromes which cause deficiency of fat soluble vitamins, especially vitamin E, suggesting association with injury of mitochondrial membrane by oxidative stress [9]. In the present case, blood vitamin E concentration was not measured. Histopathologically, no findings that could have caused wasting, gastrointestinal symptoms or malnutrition were observed. Atrophy of villi and inflammatory cell infiltration were observed in the small intestine, but these changes were not related because they were mild in the present case and they are common in cynomolgus monkeys showing chronic wasting of unknown cause.

Based on the characteristics of the distribution and severity of the lesion, we diagnosed the present case as vascular smooth muscle lipofuscinosis. A hallmark of the lipofuscinosis of the case is lipofuscin deposition in systemic vascular smooth muscles, occurring predominantly in the veins; however, there was no tendency of deposition in specific organ systems. To our knowledge, this is the first report on vascular smooth muscle lipofuscinosis occurring predominantly in the veins of primates and other animals.

CONFLICT OF INTEREST. The authors have no conflicts of interest directly relevant to the content of this article.

ACKNOWLEDGMENTS. We thank Mr. Hideyuki Watanabe for excellent technical assistance and Mr. James Harada and Ms. Kanae Tamatsukuri for help with language editing.

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