Comparative Clinical Pathology (2019) 28:705–709
https://doi.org/10.1007/s00580-019-02951-w

CASE REPORT

Systemic lipofuscinosis associated with a lesion of autophagic vacuolar myopathy in the diaphragmatic muscle of a cow

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Received: 8 January 2019 / Accepted: 4 April 2019 / Published online: 16 April 2019

Abstract

A 7-year-old Holstein-Friesian dairy cow that was culled because of traumatic motor dysfunction due to accidental falling down showed a variety of gross postmortem findings, such as fracture of the caudal vertebra, massive hemorrhage in the pelvic cavity, edematous swelling of the right sciatic nerve, and brown discoloration of the masseter muscle, tongue, cardiac muscle, diaphragmatic muscle, liver, adrenal cortex, and intestinal wall. Histopathologically, the discolored and some other internal organs/tissues exhibited varying degrees of intracellular deposition of lipofuscin granules. A small number of eosinophilic proteinaceous granules, along with lipofuscin granules, were found in the smooth muscle fibers of the intestinal muscular layer. The diaphragmatic muscle showed a lesion of autophagic vacuolar myopathy, representing frequent occurrence of autophagic vacuoles that contained amorphous materials, lipofuscin granules, and eosinophilic proteinaceous droplets. It is presumable that the pathogenetic mechanism of this systemic lipofuscinosis had a close relationship with ischemia due to anemic condition caused by pelvic hemorrhage. This bovine case was considered a newly identified variant of systemic lipofuscinosis associated with an autophagic vacuolar lesion in the diaphragmatic muscle.

Keywords Autophagic vacuoles · Cattle · Lipofuscin · Muscle · Proteinaceous substances

Introduction

Dark pigmentation due to deposition of lipofuscin granules (LGs) occurs in muscle tissues of domestic animals, including cardiac, skeletal, or smooth muscle of the bovine species (Bradley and Duffell 1982; Jolly 1995; McGavin 1995; Myers et al. 2012; Ohfuji 2015; Valentine 2017). It has been acknowledged that the autophagy system is required for basal muscle fiber homeostasis in skeletal muscle (Nishino 2010; Sandri 2010). Autophagic vacuoles (AVs) are known to be a frequent feature in a group of muscle disorders referred to as autophagic vacuolar myopathies (AVM) in humans, including Pompe disease, Danon disease, and X-linked myopathy with excessive autophagy (Sugie et al. 2005; Nishino 2003; Nishino 2006; Malicdan et al. 2008; Grumati and Bonaldo 2012). Recent studies by using molecular genetic techniques have revealed that a heterozygous CLN3 mutation is associated with juvenile onset neuronal ceroid-lipofuscinosis and AVM in humans (Licchetta et al. 2015; Taschner et al. 2015; Radke et al. 2018). Lesions of AVM have scarcely been described in domestic animals including the bovine species. The present report describes the histopathological findings in a bovine case of systemic lipofuscinosis associated with a vacuolar lesion similar to that of AVM in the diaphragmatic muscle.

Case description

A 7-year-old Holstein-Friesian dairy cow that had been raised in a local dairy farm fell down accidentally in a cow barn. Immediately thereafter, the animal showed clinical signs of motor dysfunction, such as difficulty in rising, inability to rise, and ultimately sternal recumbency. There were no clinical signs indicative of those seen in the central nervous system (CNS) disorder, such as loss of consciousness, convulsion, and abnormal behavior. Because of poor prognosis determined by the referring veterinarian, the owner decided to cull the cow. The animal was slaughtered at a public abattoir 5 days after the onset of clinical sign. After postmortem inspection, tissue samples for histopathological examination were taken

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from various internal visceral organs and tissues, including the liver, spleen, kidney, heart, lung, adrenal gland, small intestine, tongue, masseter muscle, diaphragmatic muscle, semimembranosus muscle, semitendinosus muscle, and medulla oblongata. These samples were fixed in 10% neutral buffered formalin for 72 h, processed by routine methods, and embedded in paraffin wax. Four-μm-thick sections were cut and stained with hematoxylin and eosin (HE). Selected sections were stained with periodic acid-Schiff (PAS), Masson's trichrome, luxol fast blue (LFB)-HE, phosphotungstic acid-hematoxylin (PTAH), Prussian blue, and Sudan black B. Selected unstained paraffin-embedded sections were examined with fluorescence microscopy under ultraviolet light.

Results

On postmortem inspection, the present cow exhibited fracture of the caudal vertebra, extensive hemorrhage in the pelvic cavity, and edematous swelling of the right sciatic nerve. In addition, brown discoloration of the masseter muscle, tongue, cardiac muscle, diaphragmatic muscle, liver, adrenal cortex, and wall of the small intestine was noted. Other internal visceral organs and tissues were generally unremarkable.

Histopathologically, this cow exhibited a pigmented condition of systemic lipofuscinosis. Although to varying degrees, deposition of LGs was found in a wide variety of otherwise normal cells, including the hepatocytes, renal tubular epithelial cells, cardiac muscle fibers, cardiac Purkinje fibers, adrenocortical cells, striated muscle fibers (the masseter muscle, tongue, diaphragmatic muscle, semimembranosus muscle, and semitendinosus muscle), and smooth muscle fibers in the muscular layer of small intestine (Fig. 1). In the medulla oblongata nuclei, LGs were deposited within neurons and glial cells that exhibited no significant increase in number. LGs stained yellowish brown with HE and Masson's trichrome, positively with PAS, dark blue with LFB-HE, pale blue with PTAH, negatively with Prussian blue, and black with Sudan black B (Fig. 2, inset). LGs showed yellow-orange autofluorescence emission with fluorescence microscopy.

Along with LGs, a small number of eosinophilic proteinaceous granules (EPGs) were found in the smooth muscle fibers of the intestinal muscular layer (Fig. 2). These EPGs were scattered randomly in the smooth muscle fibers. In addition, the diaphragmatic muscle often had clear sarcoplasmic vacuoles (< 30 μm in diameter) that were consistent with AVs. In many instances, these AVs were situated at the deeper or central portion of each muscle fiber, occurring singly per muscle fiber. Some AVs contained amorphous materials together with varying amounts of LGs (Fig. 3). Eosinophilic proteinaceous droplets (EPDs) that exhibited a hyaline appearance and were up to 10 μm in diameter were rarely found within the AVs (Fig. 4). Although both EPGs and EPDs stained negatively with PAS, LFB-HE, Prussian blue, and Sudan black B, they stained blue with PTAH and orange-red with Masson's trichrome. No autofluorescence emission was recognized in EPGs and EPDs with fluorescence microscopy.

Changes in other internal visceral organs included a mild infiltration of lymphocytes and siderophages in the interlobular connective tissue of the liver and prominent splenic hemosiderosis.

Discussion

On the basis of the histochemical findings and fluorescent characteristics, the intracellular pigment granules recognized in a wide variety of internal visceral organs and tissues of the present cow were identical to LGs. This pigmented condition, diagnosed as systemic lipofuscinosis, was different from an inherited neurodegenerative disorder of bovine ceroid-

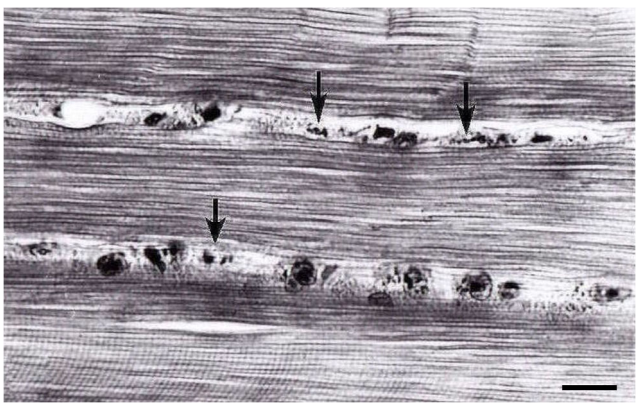

Fig. 1 Many lipofuscin granules (*arrows*) are seen in the perinuclear areas of the masseter muscle. HE. Bar = 28 μm

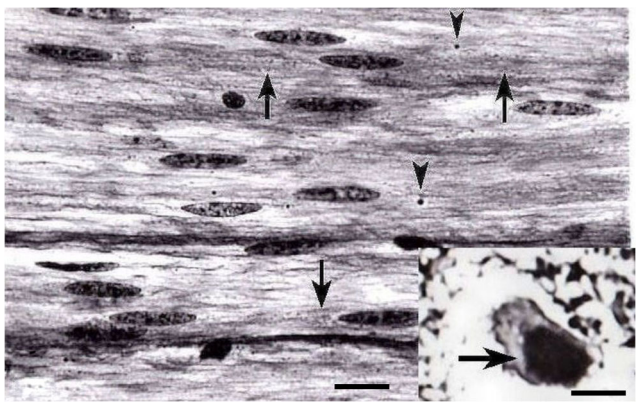

Fig. 2 Smooth muscle fibers in the muscular layer of small intestine exhibit deposition of lipofuscin granules (*arrows*) and EPGs (*arrowheads*). HE. Bar = 28 μm. *Inset* shows deposition of lipofuscin granules (*arrow*) in a neuron of the medulla oblongata. Sudan black B. Bar = 28 μm

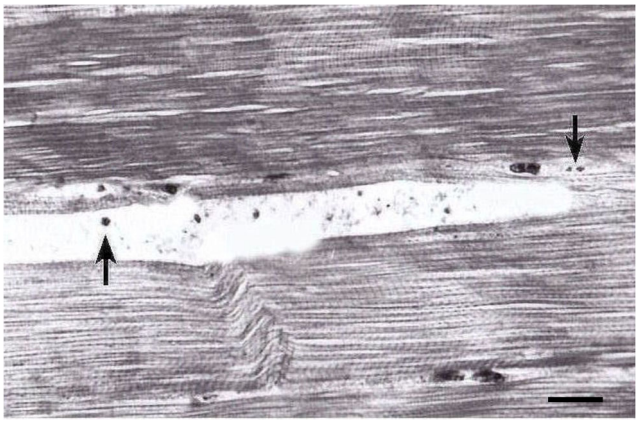

Fig. 3 LGs (*arrows*) are found within an autophagic vacuole and sarcoplasm of muscle fiber in the diaphragmatic muscle. HE. Bar = 28 μm

lipofuscinosis in as much as it lacked neurological clinical signs, such as dementia, blindness, and ataxia. Bovine ceroid-lipofuscinosis, a disorder characterized by lysosomal storage of a fluorescent lipopigment within a variety of cells, is histopathologically associated with elective necrosis/loss of neurons and astrocytosis throughout the CNS (Jolly et al. 1992; Jubb and Huxtable 1993; Jolly and Walkley 1997; Hafner et al. 2005; Houweling et al. 2006). These neural lesions were absent in the medulla oblongata of the present cow. Although the precise risk factor for the development of this systemic lipofuscinois could not be determined, it should be noted that the animal had anemia due to massive hemorrhage involving the pelvic cavity. Therefore, there is the likelihood that the ischemic insult associated with anemic condition had a close relationship with the pathogenesis of such a variant of lipofuscinosis. Indeed, previous studies have verified sufficient evidence that ischemia is pathogenetically associated with accumulation of LGs in various organs and tissues, including cardiac muscle fibers of young rabbits with acute myocardial infarction (Tatariunas 1991), black esophagus

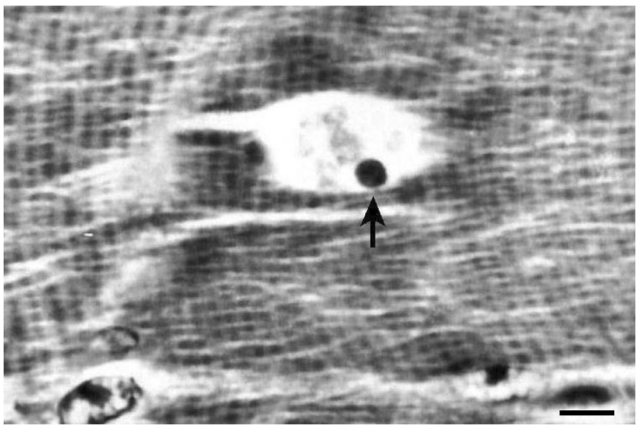

Fig. 4 An eosinophilic proteinaceous droplet (*arrow*) with amorphous materials is seen within an autophagic vacuole in muscle fiber of the diaphragmatic muscle. Masson's trichrome. Bar = 18 μm

with ischemic necrosis of the mucosa, submucosa, and muscularis propria in humans (Altenburger et al. 2011), and brain or spiral ganglion following global brain ischemia in experimental animals (Lukan et al. 2000; Ohtaki et al. 2012). Some authors thought that the incidence of LGs is related to the peroxidative membrane damage due to ischemia (Lukan et al. 2000).

As is the case with the present cow, intracellular accumulations of proteins on HE-stained sections usually exhibit a hyaline (homogenous, eosinophilic, and translucent) appearance (Miller and Zachary 2017) or likewise appear as rounded, eosinophilic droplets (Kumar et al. 2015). Both the LGs and EPGs (or EPDs) were present within the same muscle fibers in this cow, suggesting that these morphologically different types of intrasarcoplasmic deposits might have been metabolically related to each other. This possibility may be substantiated, in part, by the previous studies which have revealed that lipofuscin consists of a variety of chemical composition, including lipids, proteins, dolichols, carbohydrates, and metals (Jolly et al. 1995; MacLachlan and Cullen 1995; Miller and Zachary 2017).

The diaphragmatic muscle of the present cow noticeably exhibited a vacuolar lesion similar to that seen in AVM in humans. This vacuolar lesion, characterized by intrasarcoplasmic occurrence of many AVs, was differentiated from that reported previously in a genetic/metabolic disorder of generalized glycogenosis in cattle (Howell et al. 1981; Zlotowski et al. 2006; Cítek et al. 2007) on the basis of the absence of PAS-positive substances in the sarcoplasmic AVs. In addition, the presence of LGs and EPDs within the AVs excluded the likelihood of another type of vacuolation (hydropic degeneration) that is known as a less common, non-specific feature of myopathies (Hulland 1993; Berridge et al. 2018).

It is acknowledged that lysosomes play an important role in the autophagic pathway by digesting their contents (Lieberman et al. 2012) and that some fragments of cell components digested by lysosomal enzymes persist within AVs as lipofuscin (Grune et al. 2001; Miller and Zachary 2017). Furthermore, autophagy activation is critical for protein breakdown in skeletal muscle (Sandri 2010), and proteins are known to be major targets of oxidative modification (Grune et al. 2001). The balance between the protein damage and clearance of damaged proteins is disturbed under stress conditions, which may lead to a malfunctioning of proteostasis and an accumulating mass of oxidized proteins, finally resulting in the accumulation of highly cross-linked materials such as lipofuscin (Höhn and Grune 2013). Therefore, it is probable that in the diaphragmatic muscle of the present cow, the microscopically visible retention of EPDs within AVs as well as the identification of pigmented AVs may imply incomplete degradation of proteins and lipids (Sulzer

et al. 2008), suggesting an impairment or block of autophagic flux (Sandri 2010; Lieberman et al. 2012). Although autophagy has been invoked as a mechanism of cell loss in degenerative diseases of muscle (Kumar et al. 2015), this cow's diaphragmatic muscle exhibited no significant changes other than intrasarcoplasmic deposition of LGs, as in many other muscle tissues. Therefore, it remains to be accounted for why excessive development of AVs was restricted to this respiratory muscle.

In conclusion, this bovine case was considered a newly identified variant of systemic lipofuscinosis associated with an autophagic vacuolar lesion in the diaphragmatic muscle. Further research by using more specific methodologies, such as ultrastructural investigations and molecular genetic techniques, would be warranted for more accurate understanding of the pathophysiological mechanism for eliciting this condition in the bovine species.

Compliance with ethical standards

Conflict of interest The authors declare that they have no competing interests.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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