Pulmonary Dystrophic Oxalosis and its Possible Relation to Fibrosis in an Aged Gentoo Penguin (Pygoscelis papua)

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ABSTRACT. A 20-year-old Gentoo penguin was found dead with a clinical history of inappetence and dyspnoea. At necropsy, the lungs showed severe congestion/hemorrhage and atelectasis. Histopathologically, fibrosis was observed exclusively around parabronchi with severe collagen deposition. In fibrotic lesions, there were numerous depositions of crystalline structures accompanied by epithelioid cells and multinucleated giant cells (foreign body type). In addition to irregularly lamellar structures as the morphology, the crystals were demonstrated as precipitated calcium oxalate (CaOx) by the Alizarin red S staining with and without polarized light and von Kossa’s staining. Mycobacteria and fungi were not found by special and immuno-histochemical stainings. Pulmonary dystrophic oxalosis is a very rare lesion in Gentoo penguin.

Key words: calcium oxalate, dystrophic oxalate, gentoo penguin, lung.

Oxalosis is characterized by accumulation of crystalline deposits of insoluble calcium oxalate (CaOx). The pathological conditions of oxalosis may be hereditary or acquired, that should be developed while young. In humans, the hereditary oxalosis occurs as a result of autosomal recessive disorder in which there is abnormality of glyoxylate metabolism resulting in excess production of oxalate ions and increased absorption of oxalate ions by the gastrointestinal tract [3]. In veterinary medicine, there are a few reports on hereditary renal oxalosis in cats [26]. In humans, on the other hand, the acquired oxalosis includes 5 categories; due to excess ingestion of oxalate or oxalate producing compounds (exogenous oxalosis): increased absorption of normal dietary oxalate in enteric diseases (enteric oxalosis): oxalosis associated with chronic renal insufficiency (uremic oxalosis): excessive intake of vitamin C, and dietary insufficiency of pyridoxine (deficiency oxalosis) [3, 6, 22]. In hereditary and acquired oxalosis, the crystalline depositions are seen systemically in the kidneys, bone, myocardium and thyroid glands, being accompanied with increased concentration of oxalate in body fluids and hyperoxaluria [6]. Besides these systemic oxalosis, dystrophic oxalosis, which is defined as CaOx deposition without changes in systemic oxalate metabolism, exists [3]. The dystrophic oxalosis may be associated with tissue injury/inflammation or infection by oxalate producing micro-organisms such as Aspergillus niger [3, 10]; some human cases of pulmonary oxalosis have been reported to be secondary to A. niger infection [7, 23]. Dystrophic oxalosis has been reported in degenerative tissues of eyes and kidneys in human patients with acquired immunodeficiency syndrome (AIDS) [20, 21]. We encountered a lot of deposition of oxalate crystals in fibrotic lesions of the lungs of an aged penguin. Based on intensive pathological examinations, the condition was considered pulmonary dystrophic oxalosis, which is the first case in Gentoo penguin.

A 20-year-old, male Gentoo penguin (Pygoscelis papua), kept as an exhibit in a Zoo (Wakayama, Japan), was found dead after 1-week-clinical history of inappetence and hyperpnoea. At necropsy, severe congestion/hemorrhage and atelectasis were observed in the lung (Fig. 1). Major organs were fixed in 10% neutral buffered formalin for histopathological examinations. Paraffin-embedded tissues were cut at 4 µm in thickness. Besides hematoxylin and eosin (HE) stain, deparaffinized sections were stained with Periodic acid-Schiff (PAS) reaction, azan-Mallory stain, von Kossa’s stain, Grocott’s Methenamine Silver (GMS) stain, Alizarin red S stain (sections were pre-treated with 2M acetic acid for 20 min), sections were treated with 3% H2O2 in phosphate buffered saline (PBS) to quench endogenous peroxidase activity and then with 5% skimmed milk to inhibit non-specific reactions. The sections were incubated with each primary antibody overnight at 4°C and then reacted with labeled secondary antibody (Histofine Simple Stain MAX PO, Nichirei, Tokyo, Japan). Positive reactions were visualized with 3, 3’-diaminobenzidine (DAB Substrate Kit, Vector Laboratories Inc., CA, U.S.A.). Bovine pulmonary aspergillosis and chelonian intestinal candidiasis [12] were used as positive controls. Non-immunized rabbit serum was used as a negative control. Sections were counter-
stained lightly with hematoxylin.

Besides congestion and hemorrhage, fibrotic lesions were seen in the lungs, exclusively around the parabronchi with severe collagen deposition (Fig. 2). The severity of fibrosis was demonstrated by the azan-Mallory stain (Fig. 3). In HE-stained sections, furthermore, multifocal depositions of faintly or strongly basophilic materials forming lamellar crystalline structures were observed in the fibrotic lesions (Fig. 2). Examinations with polarized light in HE-stained sections revealed the deposition of birefringent crystals (Fig. 4). In the fibrotic area, interestingly, many multinucleated giant cells and epithelioid cells were seen focally or diffusely (Fig. 2). Multinucleated giant cells and epithelioid cells often surrounded the crystalline structures, and some of them phagocytized the crystalline debris (Fig. 2; right-side inset). Crystalline structures were stained black with von Kossa’s staining in varying degrees, indicating the inclusion of various amounts of calcium (Fig. 5). Bright orange color birefringence was observed with partially polarized light in Alizarin red S-stained sections (Fig. 6). Alizarin red S staining with and without acetic acid treatment revealed that more than 80% of crystals consisted of CaOx. Materials reactive by the PAS reaction were not seen in the crystals. Heterophils and lymphocytes were rarely seen in the fibrotic areas. Consecutive and extensive histopathological sections, which were stained with HE stain and by immunohistochemistry for Aspergillus spp. and Candida spp., did not reveal any presence of infectious fungi. Additionally, the PAS/GMS stains and Ziehl-Neelsen stain for fungi and mycobacteria, respectively, gave negative results for lung sections.

In other organs such as the kidneys, liver, heart, pancreas, spleen, brain and intestines, neither significant histopathological findings nor micro-organisms were found; CaOx crystals were not found in these organs.

Oxalate is a metabolic end product of plants and has no benefit to animal body [6]. CaOx crystals seen in animals have various morphology such as rosette, lamellar, dumbbell and sheaf-like shapes and show strong birefringent with partially polarized light in HE-stained sections [7, 9, 22]. The crystalline structures seen in the present case showed the almost similar morphological findings, and a constituent of calcium salt was demonstrated by the von Kossa’s stain, indicating that the crystals are CaOx.

Besides hereditary oxalosis, systemic oxalosis may be caused by conditions such as ethylene glycol poisoning, excessive supplementation with vitamin C and excessive consumption of oxalate rich feed, leading to an increased oxalate concentration in body fluids [6, 22]. In such cases, excessive oxalate load is cleared through the kidney; therefore, hyperoxalemia is commonly associated with hyperoxaluria, and oxalate calculi are developed in the affected kidneys [6]. In the present case, there were no significant lesions nor oxalate deposition in the kidneys.

Because of the appearance of multinucleated giant cells and epithelioid cells, the infections by fungi and mycobacteria, that they can form granulomas as a specific lesion, were suspected for the present case. The Ziehl-Neelsen stain gave a negative result to mycobacterium infection; caseous necrosis, indicative of tuberculosis [27], was not seen in the present case. In humans, pulmonary oxalosis has been reported in A. niger-infected patients; the pulmonary lesions were characterized by extensive suppurative necrotizing granulomas with fungi in the center of the lesions, and CaOx depositions were seen among hyphae in some cases of A. niger-infected patients [9, 16]. In veterinary medicine, to our knowledge, cases of pulmonary oxalate crystal production in association with A. niger was reported in an alpaca [19] and in a great horned owl [28]. The lung lesions in these animals were similar to those in human cases, with abundant neutrophilic and heterophilic responses, respectively. The penguin is well known to have high sensitivity to aspergillosis particularly due to A. fumigatus, although the fungus does not produce oxalates [1, 4]. The special stainings such as PAS and GMS stains, as well as immunohistochemistry for Aspergillus spp. and Candida spp., did not reveal any infection of fungi. Granulomatous lesions formed in lungs of birds undergoing aspergillosis are characterized by a central necrosis containing numerous hyphae (and sporangium in A. fumigatus infection) surrounded by heterophils, lymphocytes, macrophages and giant cells [4]. In the present case, lesions reminiscent of suppurative necrotizing granulomas and resultant cavity formation were never found in the lungs and other organs. Except for multinucleated giant cells and epithelioid cells, inflammatory cells such as heterophils and lymphocytes were rarely seen. Based on these findings, the association with infectious agents was denied for the present case.

Pulmonary alveolar microlithiasis (PAM), a rare condition in humans and animals, is characterized by calcium phosphate microliths (calcospherites) formed within the alveolar space without inflammatory response [17, 25]. The calcospherites in PAM cases are PAS-positive amorphous to laminated concretions, and appear non-birefringent [25]. In contrast, CaOx crystals in our case were seen exclusively in the parabronchial fibrotic areas with a few inflammatory cells, showed birefringence and were negative for the PAS reaction. The PAM as a differential diagnosis was ruled out.

Healthy individuals produce around 80–90% of oxalate endogenously as a metabolic product in the degradation of glycine, glycoxylate and ascorbic acid in the liver; the metabolic product is excreted through urine [2]. Oxalates may be produced endogenously in the degradation of glycine, an important constituent of collagens and elastins [13, 14]; histopathologically, the products are infrequently accumulated as CaOx in fibrotic kidneys of animals [18]. Furthermore, an in vitro study revealed that collagen facilitates formation of CaOx crystals by acting as a matrix [15]. Previously, a case of cardiac fibrosis associated with CaOx deposition was reported in a human patient [5]. Because the present case had fibrosis in the lungs, it was considered that CaOx depositions would be related to development of fibrotic lesions, as a result of abnormal turnover of collagens. CaOx crystals themselves are able to cause severe tissue damage, and tissue damages with CaOx crystals may be further accelerated through enzymes and cytotoxic factors released from inflammatory cells, particularly reactive macrophages [8]. Multinucleated giant cells and epithelioid cells were considered to be reactive to the crystal deposition, because...
these giant cells are the foreign body type. Tissue damage induced by CaOx depositions could be enough to cause fatal pulmonary hemorrhage [16, 18]. Fibrosis and congestion/hemorrhage in the lung might be the cause of death for the present case, in addition to aging. The longevity of penguin is around 25 to 30 years [24]. On the basis of these findings,

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Fig. 1. Gross finding of formalin-fixed lung; atelectasis is seen on the cut surface. Arrows show severe fibrotic lesions. Bar=1 cm.

Fig. 2. Besides congestion and hemorrhage, fibrosis is seen particularly around the parabronchi (asterisk), with many depositions of crystalline structures (long arrows). Left inset; a crystal showing irregularly lamellar structures (arrows). Right inset; multinucleated giant cells phagocytizing crystals. HE stain. Bar=100 µm. Insets, Bar=200 µm.

Fig. 3. Depositions of CaOx crystals (arrows) are seen exclusively in the fibrotic lesion (asterisk). The azan-Mallory stain. Bar=200 µm.

Fig. 4. Crystalline structures show birefringence (arrows) with partially polarized light. HE stain. Bar=200 µm.

Fig. 5. Crystalline deposits (arrows) stain black, indicating the presence of calcium salt. von Kossa’s stain. Bar=200 µm.

Fig. 6. Crystalline structures (arrows) show bright orange birefringence with partially polarized light. Alizarin red S stain. Bar=200 µm.
the present pulmonary lesion was diagnosed as dystrophic oxalosis, presumably in association with fibrotic lesions.

In conclusion, we reported a case of pulmonary dystrophic oxalosis in an aged male Gentoo penguin, being accompanied by reactive multinucleated giant cells and epithelioid cells. The oxalosis was demonstrated as CaOx crystals. Because fibrosis was present in the lungs and oxalates are haphazardly produced endogenously in the degradation of glycine (a component of collagen) [13, 14], CaOx deposition might have been related to abnormal collagen metabolism. This is the first report of pulmonary dystrophic oxalosis in species of the Gentoo penguin. Recently, deposition of CaOx crystals within atherosclerotic plaques in coronary arteries, a site of oxalate deposition not previously found, was reported in humans; the pathogenesis remains undetermined [10]. On the other hand, dystrophic calcification is seen in necrotic and degenerating tissues, and osteopontin and osteonectin, which are locally produced, contribute to the calcification [11]. The mechanisms of dystrophic oxalosis in degenerative tissues should be investigated further by accumulating similar cases.

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REFERENCES


