Ochronosis-like condition in a cat

Laura K. Bryan*, Brad R. Weeks*, Harold Ross Payne*, Lori A. Thompson† and Joanne L. Mansell*

*Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, 4467 TAMU, College Station, TX 77843-4467, USA
†Animal Dermatology Clinic, 3901 East 82nd Street, Indianapolis, IN 46240, USA

Correspondence: Laura K. Bryan, Department of Veterinary Pathobiology, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, 4467 TAMU, College Station, TX 77843-4467, USA. E-mail: lbryan@cvm.tamu.edu

Background – Endogenous ochronosis is caused by a defect in the enzyme homogentisate 1,2-dioxygenase (HGD), which results in abnormal pigment deposition in the skin and urine abnormalities. Ochronosis previously has not been described histologically or ultrastructurally in a domestic animal species.

Hypothesis/Objectives – To describe the clinical, histopathological and ultrastructural findings in a case of aberrant pigmentation in a cat with features that resemble ochronosis.

Animal – A 5-year-old, spayed female Domestic short hair cat presented with multiple black cutaneous plaques on the face and progressive lethargy. The cat’s urine turned brown when exposed to air. The familial history of the cat was unknown.

Methods – Clinical examination; histopathology, electron microscopy and mass/energy dispersive X-ray spectroscopy of tissues.

Results – Septic peritonitis and additional pigment in the spleen, intestine and lymph node were found at post-mortem examination. The pigment was determined to be an organic compound and had a similar histological appearance, staining properties, ultrastructure and composition to ochronotic pigment. No mutations were found in exons 3, 6, 8 and 13 of the <i>HGD</i> gene in the cat.

Conclusions and clinical importance – To the best of the authors’ knowledge, this is the first report of a condition resembling ochronosis in a domestic animal species that has been evaluated with histopathology and advanced imaging techniques. It provides an additional differential in cases of aberrant pigmentation in cats.

Introduction

Ochronosis is a systemic human disorder which results in black/brown pigment deposition in the skin, periarticular connective tissues, cartilage and heart valves.1,2 The endogenous form of ochronosis is caused by alkaptonuria, an autosomal recessive condition that results from a defect in the metabolism of homogentisic acid (HGA), and causes urine to turn dark brown when exposed to air or sunlight.1,3 The term “ochronosis” was first coined by Rudolf Virchow in 1866.4 Failure to metabolize HGA, an intermediate product of phenylalanine and tyrosine metabolism, causes alkaptonuria and consequently ochronosis.1,2,4 HGA is oxidized by homogentisate 1,2-dioxygenase (HGD) to be used as a substrate in the citric acid cycle.2 When HGD is absent or nonfunctional, a melanin-like pigment derivative of HGA, benzoquinoneacetate, accumulates in tissues and alters collagen cross-linking, causing joint pain and cartilage degeneration.1 Alkaptonuria is rare in humans, occurring in one of 250,000 to 1 million births.4 Deleterious mutations, usually missense or splice site mutations, can occur within all 14 exons and in intron regions of the 60 kb <i>HGD</i> gene.4 Aside from a murine model, ochronosis has not been definitively documented in a domestic animal species.5

Case report

A 5-year-old, spayed, female domestic short hair cat was presented for evaluation of multiple black cutaneous plaques (Figure 1) over the muzzle, pinnae and ear canal, with extension into the conjunctiva, oral mucosa and tonsil. The cat’s familial history was unknown, and the cat was negative for feline leukaemia and immunodeficiency virus. The skin lesions on the pinnae were first observed when the cat was a kitten and the plaques had gradually progressed in size and severity over time. Initial biopsies of the lesions returned a diagnosis of “calcinosis circumscripta.” The referring veterinarian had treated the skin lesions with methylprednisolone injections every 30–60 days for 2 years, but the plaques had not resolved. On physical examination, the plaques were nonpainful, firm and hairless. Biopsies from the skin lesions and oral mucosa were submitted for histopathology. The skin lesions did not resolve and the cat became increasingly...
lethargic and anorexic. Urine collected from the cat turned dark brown when exposed to air and sunlight. Approximately 2 months after initial presentation, the cat declined and euthanasia was elected. The referring veterinarian performed a cosmetic postmortem examination (PME) and discovered a 6.94 cm, spongy, tan-black mass within the mesentery. Dozens of small, black nodules were on the margins of the spleen, within the intestinal serosa and a mesenteric lymph node. Formalin-fixed sections of the abdominal mass, skin, oral mucosa, spleen, mesenteric lymph node and jejunum were submitted for histopathology.

The tissues were processed for routine paraffin embedding and stained with haematoxylin and eosin. Histology of the skin and oral mucosa from the pre- and post-mortem biopsies revealed dozens of islands, 100–500 μm in diameter, of extracellular dark brown to black, granular pigmented material within the deep dermis, panniculus and oral submucosa (Figure 2a). The pigment was often arranged in lamellar aggregates (Figure 2b) surrounded by macrophages and multinucleated giant cells. The pigment was not associated with hair follicles or other adnexal structures. Granulomatous inflammation surrounding similar aggregates of pigment was also within the muscle of a segment of jejunum (Figure 2c), within the splenic red pulp and the sinuses of a mesenteric lymph node (Figure S1 in Supporting information). The abdominal mass noted grossly was a granuloma containing pigment and mixed bacteria (septic granulomatous peritonitis). Bacteria were observed only in the abdominal mass. The pigment was positive staining with Fontana–Masson and Schmorl’s ferricyanide reducing stains (Figure S2 a,b in Supporting information) and could be removed with potassium permanganate bleaching. Prussian blue and rhodanine stains for iron and copper, respectively, were negative.

Sections of formalin-fixed skin were processed routinely for mass spectroscopy, transmission electron microscopy (TEM) and low-vacuum scanning electron microscopy (SEM) with energy-dispersive X-ray spectroscopy (EDS) instrumentation. The pigment was determined to be an organic compound with a projected 14-carbon backbone with no evidence of polymer formation, but detailed mass spectroscopic analysis of the pigment was hindered by prolonged formalin fixation. On TEM, vast deposits of electron-dense, amorphous material were between collagen fibres in the dermis (Figure 3). Similar pigment was found within the cytoplasm of macrophages; on SEM imaging, the pigment was...
deposited in concentric whorls (Figure 4a). EDS analysis of the pigment revealed a large proportion of sulfur and phosphorus with smaller foci containing sodium, carbon and oxygen (Figure 4b).

DNA was extracted using a BiOstic® kit (MO-BIO Labs; Carlsbad, CA, USA) from formalin-fixed paraffin embedded (FFPE) tissue and from three dermatologically normal feline cadavers euthanized for other clinical conditions. The feline equivalent of exons 3, 6, 8 and 13 of the HGD gene, the most common sites of mutation in humans, were analysed via PCR through creation of flanking primers (see Table S1 in Supporting information). Amplified DNA bands were sequenced (Eton Biosciences; San Diego, CA, USA) and analysed with BLAST-n (NCBI; Bethesda, MD, USA) and Clustal Omega (EMBL-EBI; Hinxton, UK). There were no differences between the reference sequence and the affected and control cat DNA sequences for the examined HGD exons.

Discussion

Based on the histological characteristics of the pigment and the clinically observed delayed urine colour change in the cat, the pigment was identified as a melanin-like substance and a possible derivative of HGA. This is the first histologically and ultrastructurally confirmed report of an ochronosis-like condition in a domestic animal species. The full extent of pigment deposition in the cat is unknown due to the constraints of a cosmetic PME and the classic sites of ochronotic pigment deposition, such as periauricular tissues, heart valves and bones, were not examined. The inciting cause of the abdominal mass was not evident histologically, but the lesions in the section of intact jejunum were suggestive of a foreign body-type reaction to the pigment. The chronic inflammation induced by the accumulated pigment most likely weakened the wall of another intestinal segment, causing septic peritonitis and the cat’s worsening clinical signs. Bacteria were observed only within the abdominal mass, making production of the pigment by microbes unlikely. The cutaneous plaques had also been present since the cat was a kitten, suggesting that the aberrant pigmentation was more likely secondary to a genetic condition than other factors in the clinical history, such as exposure to hydroquinone-containing skin creams, which could be a cause of acquired ochronosis.

Differential diagnoses for extracellular, brown-black pigment accumulation in tissue include congenital porphyria, melanosis and ochronosis. Haemochromatosis and copper storage disorders were ruled out based on the results of histological stains. Congenital porphyria has rarely been reported in cats and typically results in diffuse orange-yellow to occasionally brown pigmentation of bones and teeth with a persistent red-tinge to urine, but has not been reported to cause discrete pigment aggregates in skin or viscera.6,7 Porphyria is an unlikely factor in the present case due to lack of discoloured teeth and urine colour change only when exposed to air. Melanosis and

Figure 3. Ochronosis-like condition in a cat. Transmission electron microscopy of the skin. Pigment globules (arrows) were within the cytoplasm of a macrophage, whereas extracellular pigment was electron dense and amorphous (asterisk), bar = 5 μm.

Figure 4. Ochronosis-like condition in a cat. (a) Scanning electron microscopy (SEM) of the pigmented areas. Revealed that the pigment was predominantly extracellular and arranged in concentric whorls, bar = 100 μm. (b) Energy-dispersive X-ray spectroscopy (EDS) instrumentation on the SEM field depicted in Figure 4a revealed a large proportion of sulfur (turquoise) and phosphorus (pink) with smaller foci containing sodium (blue), carbon (red) and oxygen (green) in the pigment; BSE, backscattered electrons.
Ochronosis were once considered the same entity in the early 20th Century and cause similar pigmentation of bones and joints. Pigmenturia is not associated with melanosis, but is a cardinal feature of endogenous ochronosis. Rare cases of black pigmentation in the bones of cattle and pigs have been described, but skin lesions were not reported and additional diagnostic tests were not performed to distinguish ochronosis from melanosis. Although pigment deposition outside the skin, periarticular connective tissues and heart valves is uncommon in human cases of ochronosis, mice routinely develop large pigment aggregates in the kidneys and liver in the model for alkaptonuria which suggests that the pigment deposition site may be different in this condition.

The gold standard for diagnosis of ochronosis is analysis of the urine for HGA, however, unfortunately, urine was not submitted for testing in this case. Histological diagnosis of ochronosis is complicated because melanin and ochronotic pigment both stain positive with Fontana–Masson and Schmorl’s technique. The TEM and SEM findings were similar to previous descriptions of ochronotic pigment in human heart valves. Ochronosis is not a fatal condition; although the symptoms of the disease can be managed through prosthetic replacement of affected joints, there is no cure or effective treatment to mitigate HGA build-up in tissues. Attempts to find a mutation in the HGD gene in the affected cat through PCR were hindered due to fragmentation of the DNA in the FFPE sample, which prevented sequencing of the entire HGD gene. Although no differences were found between the DNA sequences for HGD exons 3, 6, 8 and 13 for the affected cat, mutations may exist elsewhere in unexamined coding and noncoding regions of the HGD gene or in regulatory regions. Based on the histological and ultrastructural findings, the cat investigated in this report most likely had a genetic condition that caused aberrant skin and organ pigmentation with features similar to those observed in cases of ochronosis.

Acknowledgements

The authors would like to thank Yohannes H. Rezenom for his assistance with mass spectroscopy.

References


Supporting Information

Additional Supporting Information may be found in the online version of this article.

Figure S1. Large, brown pigment aggregates were within the subcapsular and medullary sinuses of a mesenteric lymph node, 10×, H&E.

Figure S2. The pigment stained positive (black) with Fontana-Masson reducing stain, skin, 400×, H&E.

Table S1. Conventional PCR primer sets for DNA amplification of select exons of the Feline homogentisate 1,2-dioxygenase (HGD) gene. Primers are given in the 5′ to 3′ direction and are derived from the Ensembl reference sequence ENSFCAG0000031534.
Conclusions and importance clinique – A la connaissance des auteurs, ceci est le premier cas d’animal domestique présentant une atteinte semblable à l’ochronose, évalué par histopathologie et des techniques d’imagerie avancées. Cette atteinte s’ajoute au diagnostic différentiel des cas de pigmentation anormale du chat.

Resumen

Introducción – la ochronosis endógena está causada por un defecto de la enzima dioxigenasa homogentisato 1,2 (HGD), lo cual resulta en deposición anormal de pigmento en la piel y anormalidades en la orina. La ochronosis no ha sido descrita previamente a nivel histológico ni ultrastructural en un animal doméstico.

Hipótesis/Objetivos – describir los hallazgos clínicos, histopatológicos y ultraestructurales en un caso de pigmentación aberrante en un gato con características similares ochronosis.

Animal – una hembra esterilizada de cinco años de edad de raza Doméstica de Pelo Corto se presentó con múltiples placas cutáneas de color negro en la cara y letargia progresiva. La orina del gato se volvió de color marrón cuando se exponía al aire. Se desconocía la historia familiar del gato.

Métodos – examen clínico, histopatológico, microscopía electrónica y espectросcopia de masa/energía dispersiva de rayos X de los tejidos.

Resultados – se observó una peritonitis séptica y presencia de pigmento en el bazo, intestino y ganglio durante el examen postmortem. El pigmento se determinó como un compuesto orgánico y tenía una apariencia histológica similar, propiedades de tinción, ultraestructura y composición a pigmento ochronótico. No se detectaron mutaciones en los exones 3, 6, 8 y 13 del gen HGD en el gato.

Conclusión e importancia clínica – según nuestros conocimientos, este es la primera descripción de una enfermedad similar a ochronosis en especies domésticas que haya sido evaluada con histopatología y técnicas de imagen avanzadas. Aporta otro diferencial en casos de pigmentación aberrante en gatos.
摘要
背景 — 内源性褐黄病是由尿黑酸1,2-二氧化酶(HGD)缺陷造成，进而导致皮肤色素沉积和排尿异常。此前没有关于家养动物褐黄病组织学或超微结构的描述。
假设/目的 — 只色素沉积异常且疑似褐黄病的猫，描述其临床症状、组织病理学和超微结构上的发现。
动物 — 一只五岁、已绝育的家养短毛猫，面部多处黑色斑块且渐进性嗜睡。该猫尿液遇空气变为褐色。该猫家族史不详。
方法 — 临床检查；并对组织进行组织病理学、电子显微镜和能量X-ray光谱检查。
结果 — 尸检发现败血症性腹膜炎，以及脾脏、肠道和淋巴结发现色素异常，该色素为有机化合物，和褐黄病的色素具有相似的组织学表现、染色属性、超微结构和构成。该猫未发现HGD基因的外显子3，6，8和13突变。
总结和临床意义 — 据作者所知，这是第一篇有关疑似家养动物褐黄病的报道，并且通过组织病理学和先进的影像技术进行了评估。为猫异常色素沉着的病例提供新的鉴别诊断种类。