Calcinosis Circumspecta in a Common Marmoset (Callithrix jacchus jacchus)

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A 2.5-y-old, male common marmoset (Callithrix jacchus jacchus) developed a 2-cm, interscapular, subcutaneous mass with variably firm and cystic areas. Radiographs demonstrated a radiodense mass in close proximity to a previously implanted microchip. Fine-needle aspiration yielded a chalky liquid that, on cytologic examination, contained amorphous debris. Aerobic and anaerobic cultures were negative. Surgical excision required extensive dissection, with the mass infiltrating deep to the scapula and extending to the mammary gland. The mass weighed 30 g and comprised 10% of the animal’s body weight. Microscopic examination demonstrated multifocal, variably sized, amorphous aggregates of granular, basophilic material (mineral) in the subcutis and extending to skeletal muscle. Mineral deposits were surrounded by macrophages, giant cells, and fibrous connective tissue. A focal area of ectopic bone production was present. Crystallographic analysis and x-ray diffractometry determined the material to be comprised of 100% hydroxyapatite. These findings were consistent with a diagnosis of calcinosis circumscripta. Systemic metabolic abnormalities were excluded based on examination of complete blood count, serum chemistry, and ionized calcium. Calcinosis circumscripta in the common marmoset has not previously been reported, although the lesion has been reported to occur in rhesus macaques and is well described in man and dogs. Accumulation of calcium deposits and production of ectopic bone in a marmoset is interesting in light of this species’s unique calcium and vitamin D metabolism.

Calcinosis is the deposition of calcium salts with subsequent tissue hardening, a process that occurs normally in bone and teeth. In contrast, the abnormal deposition of mineral in soft tissue is broadly termed ectopic mineralization. Calcinosis circumscripta is a form of ectopic mineralization characterized by single or multiple cutaneous nodules containing a semisolid deposition of calcium salts. This syndrome has been described in numerous species including humans,14 rhesus macaques,8 dogs,13 cats,11 horses,3 cows,1 buffalo,6 and captive sitatunga (tragelaphus spekei).17 Here we present a case of calcinosis circumscripta in a common marmoset.

Case Report

A 2.5-y-old, 300-g, male, colony-bred common marmoset (Callithrix jacchus jacchus) developed a prominent swelling in the interscapular region. This animal had been housed singly for the past year because of a history of intragroup aggression. Prior to this separation, bite wounds and abrasions were sustained. Colony housing consisted of stainless steel cages in a room maintained at 26.1 °C with 46% humidity. Diet consisted of pelleted New World Primate Diet (PMI Nutrition International, St Louis, MO) and canned ZuPreem Marmoset Diet (Premium Nutritional Products, Mission, KS) with supplements of fresh fruit and plain lowfat yogurt provided 2 or 3 times weekly. Maintenance of this colony in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care, International, was in accordance with the recommendations of the Guide for the Care and Use of Laboratory Animals.10 Historically, this animal had received neomycin injections to induce hearing loss (2.4 mg subcutaneously once daily on postnatal days 4 to 10) as part of an auditory research protocol approved by the Institutional Animal Care and Use Committee of the Johns Hopkins University.

Upon physical examination, the marmoset was bright and alert, had normal feces and urine in the cage pan, and was reported to be eating well. The animal was removed from the cage and sedated with 8 mg ketamine (Ketaset, Fort Dodge Animal Health, Fort Dodge, IA) administered intramuscularly for complete physical examination. The interscapular mass, which protruded above the dorsum and was covered by normal skin, was 2 cm in diameter, firm, and mobile (Figure 1). Upon deeper palpation, the mass extended into underlying
musculature and was composed of multifocal, fluctuant areas. A 2nd firm, nonmobile 1-cm mass was detected adjacent to the right mammary gland. This 2nd mass was suspected to be an enlarged lymph node.

Radiographs demonstrated an extensive radiodense, multilobulated mass in the subcutaneous tissue of the dorsum and axillary regions. A previously implanted microchip was located adjacent to the radiodense region (Figure 2). A fine-needle aspirate yielded a milky and slightly gritty liquid. Cytology demonstrated acellular, granular debris. Bacterial culture yielded no aerobic bacterial growth. Prophylactic antibiotic therapy (enrofloxacin, 5 mg/kg every 24 h; Baytril, Bayer Animal Health Division, Shawnee Mission, KS) was started, and surgical exploration was scheduled.

Anesthesia was induced with 8 mg ketamine and maintained with inhalant isoflurane (IsoSol, Vedco, St Joseph, MO). Buprenorphine (Buprenex, Reckitt Benckiser Pharmaceuticals, Richmond, VA) was administered preoperatively and continued postoperatively (0.003 mg subcutaneously every 12 h or more often as needed). An elliptical skin incision was made around the portion of the mass that protruded above the dorsum. The well-encapsulated mass extended from the subcutis to deep to the dorsal aspect of the left scapula and then continued ventrally to lie adjacent to the right mammary gland. Blunt dissection was performed to loosen the mass from the subcutaneous tissue and underlying musculature (cutaneous trunci and trapezius). The majority of the mass was removed via the dorsal incision site (Figure 3). The remainder of the mass was approached via a 2nd incision in the axillary region. In total, the mass weighed 30 g, equal to 10% of the marmoset’s body weight. The microchip, contained within adjacent normal subcutaneous tissue, was identified and removed as well. The muscle layers and subcutaneous tissues were apposed with 2-0 Vicryl (Ethicon, Somerville, NJ), and the skin was closed with 4-0 Prolene (Ethicon). Intraoperative samples were collected for submission of aerobic, anaerobic, and mycobacterial cultures. Aerobic culture yielded scant growth of Staphylococcus intermedius that was sensitive to a large number of antibiotics. In light of the low level of bacterial growth, this organism was likely a contaminant. Anaerobic and mycobacterial cultures yielded no growth. Enrofloxacin was continued for 7 d postoperatively.

Tissues were fixed in 10% neutral buffered formalin, embedded in paraffin, and sectioned at 4 μm. Routine hematoxylin and eosin staining revealed a mass consisting of multiple lobules of calcified material. The surrounding capsule was characterized by mature fibrous tissue containing activated fibroblasts as well as macrophages and neutrophils. Occasional macrophages contained phagocytized mineral. Numerous multinucleate giant cells also were present (Figure 4). A focal area of the mass contained trabeculae of mineralized bone and osteoid that was consistent with osseous metaplasia. Acid-fast and gram stains were negative for bacterial organisms. The morphologic diagnosis was extensive, severe dermal mineralization with chronic-active pyogranulomatous inflammation, fibrosis and osseous metaplasia. Crystallographic analysis and x-ray diffractometry of the mineralized material defined the material as 100% hydroxyapatite (Ca₅(PO₄)₃(OH)). The clinical presentation, gross findings, and histologic appearance were compatible with a diagnosis of calcinosis circumscripta.²,¹³

Blood was drawn postoperatively to determine whether any biochemical abnormalities contributed to the soft tissue calcification (Table 1). Changes in serum chemistry parameters were not clinically significant. Urinalysis demonstrated a urine pH of approximately 5.0, with trace protein and specific gravity of 1.015. Urine sediment contained rare leukocytes and transitional epithelial cells. The animal continued to do well postoperatively until euthanized 3.5 mo later as part of the research protocol. Postmortem examination identified severe, bilateral, chronic-active pyelonephritis and an additional area of subcutaneous mineralization on the ventral abdomen. There was no evidence of mineralization in internal organs such as kidney, liver, lung, or heart.

**Discussion**

The gross and histologic appearance of the calcified mass is compatible with a diagnosis of calcinosis circumscripta. Cases of calcinosis circumscripta in New World primates have not been reported previously. In man, calcinosis circumscripta...
lesions develop in the skin and subcutaneous tissues. The calcified lesions may be associated with tendon sheaths, terminal phalanges, and pressure points. Roughly 40% to 60% of cases are associated with collagen disorders such as scleroderma, Reynaud’s phenomena, and dermatomyositis. The disease is a well-documented entity of young dogs, particularly German Shepherds. In this species, the firm to fluctuant, painless nodules can range from 0.5 to 7 cm in diameter with no inflammation of overlying skin and variable amounts of alopecia. Ectopic bone often forms in calcinosis circumscripta lesions that have been mineralized for long periods of time. Recommended treatment is surgical excision, although mineral left in the lesion may serve as a focus for recurrence.

Ectopic mineral deposition in subcutaneous tissues can be divided into 3 categories: dystrophic calcification, idiopathic calcification, and metastatic calcification. The deposition of calcium salts (calcium phosphate, calcium carbonate, calcium hydroxyapatite) in damaged or degenerating tissues is termed dystrophic calcification. It is suggested that tissue damage leads to a localized decrease in cellular metabolic rate with pH increase and resultant localized precipitation of calcium and phosphorus salts. Dystrophic calcification lesions are further subdivided into localized (calcinosis circumscripta) or widespread (calcinosis universalis) lesions. Foreign bodies such as polydioxanone suture material as well as subcutaneous injection of compounds such as medroxyprogesterone and prolugosterone have been implicated as inciting causes. Degenerative skin disease (apocrine sweat gland cysts, follicular cysts) and neoplasms have also been associated with calcinosis circumscripta. In contrast, concurrent disease states including hyperglucocorticoidism and diabetes mellitus have been associated with the more widespread lesions of calcinosis universalis.

The 2nd broad category, idiopathic calcification, is defined as the deposition of calcium salts with lack of appreciable accompanying damage to tissue. Again, these lesions are subdivided into localized and widespread. Unless an etiologic agent is evident from histopathology or the clinical history, differentiation between idiopathic and dystrophic categories may be difficult.

The 3rd classification of ectopic mineralization is metastatic calcification, in which calcium salt deposition is related to systemic abnormalities in calcium and phosphorus metabolism. Demonstrable serum biochemical changes are pathognomonic. The product of calcium and phosphorus levels is often used as a prognostic indicator with products greater than 60 to 70 mg/dl associated with precipitation of calcium salts. Calcification can occur in multiple organs including lung, stomach, kidney, and vasculature. In dogs, chronic renal disease is most commonly associated with metastatic calcification. Primary and secondary hyperparathyroidism, vitamin D intoxication, and milk alkali syndrome can contribute to metastatic calcification.

The lesions in the presented case are consistent with dystrophic calcification. Events potentially leading to tissue damage in this marmoset included the reported bite wounds and microchip injection. Metastatic calcinosis was excluded from the diagnosis due to the presence of normophosphatemia, normocalcemia, and a normal ionized calcium level. Although the calcium–phosphorus product lay in the reported pathologic range for humans and dogs, no evidence of mineralization in multiple organs was found. The normal calcium–phosphorus product for the common marmoset is not reported and may be higher than that for humans and dogs. On postmortem examination, this marmoset had a severe, bilateral pyelonephritis. Urine sediment collected approximately 3 mo prior contained minimal inflammatory cells. The urine specific gravity was examined at this time and found to be in the isosthenuric range. Although prior renal insufficiency may have been present, it is unlikely the ectopic mineralization stemmed from the terminal renal pathology in light of the absence of electrolyte disturbances and azotemia when the mass was detected initially.

In a species predisposed to rachitic bone changes in the presence of inadequately formulated diets, the accumulation of calcium deposits and production of ectopic bone in this animal is surprising. The lesion was classified as a form of dystrophic mineralization, specifically calcinosis circumscripta. A diagnosis of calcinosis circumscripta should be considered as a differential for subcutaneous masses, especially those that have a mineralized character, in the common marmoset.

Acknowledgments

Crystallographic analysis was performed by Annette L Ruby and Gerald V Ling, DVM (Urinary Stone Analysis Laboratory, Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA). The ionized calcium assay was
Table 1. Serum biochemical parameters for a 2.5-y-old male common marmoset with calcinosis circumscripta at 10 d postoperatively

<table>
<thead>
<tr>
<th>Serum parameters</th>
<th>Normal range</th>
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<tr>
<td>Urea nitrogen</td>
<td>31 mg/dl; 22 ± 7 mg/dl(^a)</td>
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<tr>
<td>Creatinine</td>
<td>0.4 mg/dl; 0.6 ± 0.2 mg/dl(^b)</td>
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<tr>
<td>Glucose</td>
<td>124 mg/dl; 172 ± 48 mg/dl(^b)</td>
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<tr>
<td>Calcium</td>
<td>10.2 mg/dl, 11.1 mg/dl(^b)</td>
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<tr>
<td>Phosphorus</td>
<td>6.4 mg/dl, 5.7 mg/dl(^b)</td>
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<tr>
<td>Calcium × phosphorus</td>
<td>65.28 mg/dl, 63.27 mg/dl(^b)</td>
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<tr>
<td>Alkaline phosphatase</td>
<td>113 U/l; 61 ± 27 U/l(^b)</td>
</tr>
<tr>
<td>Ionized calcium</td>
<td>1.02 mmol/l; 1.23 ± 0.15 mmol/l(^b)</td>
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\(^a\)Mean ± 1 standard deviation; from reference 18.  
\(^b\)Value obtained at 3 wk postoperatively.  
\(^c\)Mean ± 1 standard deviation from sex- and age-matched colony controls.

performed by Patricia A Schenck, DVM, PhD, and KR Refsal, DVM, PhD (Endocrinology Section, Diagnostic Center for Population and Animal Health, Veterinary Medicine Center, Michigan State University, East Lansing, MI). Serum chemistry analysis was performed at Antech Diagnostics (Lake Success, NY). This research was supported in part by grant T32RR07002 from the National Institutes of Health.

References