Brief Report

Hypercalcemia Due to Endogenous Overproduction of Active Vitamin D in Identical Twins With Cat-Scratch Disease

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Context.—The extrarenal synthesis of active vitamin D sterols has a central causative role in the hypercalcemia associated with various granulomatous diseases.

Objective.—To study the calcium metabolism in patients with cat-scratch disease who have hypercalcemia.

Design.—Case report.

Setting.—University hospital in Barcelona, Spain.

Patients.—Two identical twin brothers who developed asymptomatic hypercalcemia during the acute phase of cat-scratch disease.

Main Outcome Measures.—Serial measures of calcium homeostasis and metabolism over a 2-month period.

Results.—On admission and 6 and 7 days later, both patients were found to have increased levels of serum and urinary calcium, serum phosphate, and serum 1,25-dihydroxyvitamin D [1,25(OH)2D], whereas they had normal values of serum 25-hydroxyvitamin D and urinary cyclic adenosine monophosphate and decreased serum concentrations of intact parathyroid hormone. Sixteen and 20 days after admission, these abnormalities had resolved without treatment. A direct correlation was observed between the serum 1,25(OH)2D levels and both the serum and 24-hour urinary calcium concentrations. Also, the concentrations of calcium and 1,25(OH)2D paralleled the clinical activity of the infectious disease over the period these parameters were measured.

Conclusions.—Our cases provide evidence that cat-scratch disease can produce hypercalcemia through the unregulated production of the metabolite 1,25(OH)2D. Cat-scratch disease should be added to the list of granuloma-forming diseases that are responsible for 1,25(OH)2D-mediated hypercalcemia.

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BARTONELLA henselae causes cat-scratch disease (CSD), meningoencephalitis, endocarditis, and prolonged fever in immunocompetent patients, and it causes bacillary angiomatosis and peliosis hepatis in patients with human immunodeficiency virus infection. The classic clinical presentation of CSD is a self-limiting regional lymphadenopathy usually caused by a cat scratch or bite. Antibiotic therapy has not been very effective in treating the disease, and in most patients it resolves spontaneously within several months. Results of routine laboratory analyses are usually normal. Rare abnormalities discovered in laboratory examinations include eosinophilia, anicteric hepatitis, and thrombocytopenia. The latter constitutes a potentially serious event.

Herein we describe 2 identical twin brothers who developed hypercalcemia, hypercalciuria, and excessively high serum concentrations of the metabolite 1,25-dihydroxyvitamin D (1,25(OH)2D) during the acute phase of CSD. The hypercalcemia in both brothers fulfilled the laboratory criteria for the diagnosis of vitamin D metabolite-mediated hypercalcemia. Furthermore, the concentrations of calcium and 1,25(OH)2D in these patients paralleled the clinical activity of the infectious disease over the period these parameters were measured.

Methods

Two identical twin brothers who were seen at our hospital in February 1997 received a diagnosis of hypercalcemia in association with CSD. Both conditions were diagnosed simultaneously in each patient. Neither had taken calcium, vitamin A, or vitamin D preparations previously. The monozygosity of the twin pair was confirmed by a dermatoglyphic analysis of fingertip prints.

Histopathologic and Microbiologic Examinations.—An excisional lymph node biopsy was performed in each patient, and the tissue specimen was divided into 2 samples for histopathologic examination and microbiologic culture. Tissue samples were stained with conventional hematoxylin-eosin as well as acid-fast, periodic acid–Schiff, and Warthin-Starry silver stains. We attempted to isolate Bartonella species from whole blood specimens and homogenized lymph node biopsy material. Specimens were inoculated both onto 5% sheep blood agar (bioMérieux, Marcy-l’Etoile, France) and into the human endothelial cell line ECV 304. Inoculated media were incubated at 37°C in a carbon dioxide atmosphere for as long as 60 days. Any isolates were identified initially by colony morphologic characteristics and negative results on tests for oxidase and catalase; identification was confirmed by species-specific mouse polyvalent antisera and by gas chromatography of whole-cell fatty acids.
Laboratory Data From Patients With Cat-Scratch Disease and Hypercalcemia

<table>
<thead>
<tr>
<th></th>
<th>Normal Range</th>
<th>Patient 1, Day</th>
<th>Patient 2, Day</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>7</td>
<td>16</td>
</tr>
<tr>
<td>Total calcium, mmol/L (mg/dL)</td>
<td>2.22-2.55 (8.9-10.2)</td>
<td>3.39 (13.6)</td>
<td>3.27 (13.1)</td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>60</td>
<td>20</td>
</tr>
<tr>
<td>Ionized calcium, mmol/L (mg/dL)</td>
<td>1.03-1.24 (4.1-4.96)</td>
<td>1.55 (6.20)</td>
<td>1.49 (5.96)</td>
</tr>
<tr>
<td>Urinary calcium, mmol/mg (mg/24 h)</td>
<td>2.60-6.06 (104-243)</td>
<td>10.78 (432)</td>
<td>9.78 (392)</td>
</tr>
<tr>
<td></td>
<td>Serum phosphorus, mmol/L (mg/dL)</td>
<td>0.81-1.29 (2.5-4.0)</td>
<td>1.52 (4.7)</td>
</tr>
<tr>
<td>Urinary calcium, mmol/mg (mg/24 h)</td>
<td>6.0-10.6 (0.4-3.5)</td>
<td>5.7 (1.9)</td>
<td>6.9 (2.3)</td>
</tr>
<tr>
<td></td>
<td>25-Hydroxyvitamin D, µmol/L (µg/mL)</td>
<td>47-161 (1-64)</td>
<td>239 (94)</td>
</tr>
<tr>
<td></td>
<td>Intact parathyroid hormone, pmol/L (pg/mL)</td>
<td>1.1-6.8 (10-65)</td>
<td>0.4 (4)</td>
</tr>
<tr>
<td></td>
<td>Urinary cyclic adenosine monophosphate, µmol/µg (mg/24 h)</td>
<td>1.2-10.6 (0.4-3.5)</td>
<td>5.7 (1.9)</td>
</tr>
</tbody>
</table>

5890 with Microbial Identification System software, version 3.0, Microbial ID Inc, Newark, Del. Lymph node specimens were also incubated for growing on conventional Lowenstein medium.

**Serologic Studies.**—The initial serum specimens were drawn within 1 week of the reported onset of illness in both patients. Serial serum samples from each patient were obtained. Serum samples were evaluated for immunoglobulin G antibodies to *B henselae* in an indirect immunofluorescent antibody assay. Antibody screening was performed according to the manufacturer’s instructions and interpreted with previously published criteria. The serologic results were reported as an end-point dilution, with a titer of 1:64 or greater considered positive (*B henselae* antibody immunofluorescence test kit, BIOS GmbH, Labordiagnostik, Munich, Germany). The demonstration of seroconversion or an increase in titer of at least 4-fold between the acute- and convalescent-phase serum specimens was considered supportive evidence of current or recent *B henselae* infection. According to the manufacturer’s catalog, no defined cross-reactivity was found when this assay was tested with serum containing antibodies to other organisms, such as *A felifis or Bartonella quintana*.

**Calcium Metabolism Studies.**—Studies of hypercalcemia included measurements of serum levels of total protein-corrected calcium, ionized calcium, serum phosphorus, intact parathyroid hormone, 25-hydroxyvitamin D, and 1,25(OH)2D. The 24-hour urinary excretions of calcium and cyclic adenosine monophosphate were also determined. These parameters were measured at different intervals over a 2-month period.

**Report of Cases**

**Patient 1.**—A previously healthy 18-year-old man was well until 5 days before evaluation, when he noted the onset of tender right cervical and preauricular lymph nodes and subjective fevers. He had purchased a 6-week-old kitten 3 weeks before the onset of his symptoms and had received multiple scratches from it since then. On physical examination his temperature was 38.3°C and he had a 5×5-cm tender right cervical lymph node as well as multiple loci of smaller, axillary, supravacuicular inguinal and left cervical lymphadenopathy. A diffuse maculopapular eruption was also observed. He had a 1.5-cm erythematous pustule on his right hand at the site of a previous scratch. Apart from an erythrocyte sedimentation rate of 64 mm/h and the abnormalities shown in the Table, results of routine laboratory examinations were normal. In addition, findings of blood cultures and serologic tests for several bacteria and viruses, including human immunodeficiency virus type 1, were negative. Circulating antibodies, anti–native DNA antibodies, and rheumatoid factor were not detected. A chest roentgenogram and an abdominal computed tomographic scan revealed no abnormalities. His symptoms abated spontaneously, and he was discharged 8 days after admission free of symptoms with no antimicrobial therapy. His cervical adenopathy resolved within 2 months.

**Patient 2.**—The identical twin brother of patient 1 presented with a 1-week history of low-grade fever, night sweats, fatigue, headache, and progressive enlargement of bulky right axillary lymphadenopathy that became painful. These symptoms began 10 days after the onset of his brother’s symptoms. He had also received several scratches from the same cat since it had been acquired. Examination revealed a temperature of 37.7°C and a 6×5-cm, tender, firm right axillary lymph node. Smaller adenopathies were found in other territories. An inoculation papule or blister was not clearly seen. Results of laboratory examinations were significant for an erythrocyte sedimentation rate of 50 mm/h, a leukocyte count of 13×10^9/L with a normal differential count, and the abnormalities shown in the Table. Results of serologic tests for several bacteria and viruses, immunologic tests, and blood cultures were all negative. Findings of a chest roentgenogram and an abdominal computed tomographic scan were normal. His symptoms decreased over a few days, and he was discharged 5 days after admission free of symptoms with no therapy. His axillary adenopathy had disappeared 3 months after admission.

**Results**

**Histopathologic Results.**—Both patients had similar histologic findings in excised lymph nodes (right cervical in patient 1 and right axillary in patient 2). Conventional hematoxylin-eosin stains were interpreted as consistent with CSD. Changes mainly consisted of lymphoid hyperplasia (that is, arteriolar proliferation, reticulum cell hyperplasia, and widening of arteriolar walls), perivascular lymphocytic infiltrates, and numerous scattered caseating granulomas containingstellate areas of necrosis with large collections of macrophages, neutrophils, and some multinucleated giant cells. No bacillary forms could be demonstrated with Warthin-Starry staining in either patient. Results of the acid-fast and periodic acid–Schiff staining procedures were also negative.

**Microbiologic Results.**—Microbiologic culture of a lymph node sample yielded growth of an organism consistent with *B henselae* after 20 days in patient 1. An isolate was also obtained from the lymph node tissue of this patient using endothelial cell coculture. Isolated colonies growing on blood agar plates had irregular morphologic characteristics and adhered to the agar surface. These colonies were small and white to gray. The identity of the isolate obtained was confirmed using *B henselae*–specific mouse polyvalent antisera and by gas chromatography of whole-cell fatty acids. Culture of a lymph node sample from patient 2 as well as cultures of whole blood specimens from both patients yielded no growth of any organism after 60 days. Finally, no isolates were obtained in either patient from lymph node biopsy material that had been incubated on Lowenstein medium after an appropriate period.

**Serologic Testing.**—Seroconversion was observed in patient 1 as late as the 12th day after onset of illness (seventh hospitalization day), with a titer of 1:256.
Antibody titers rose to 1:2048 at day 16 after admission and declined thereafter to 1:512 at 2 months after admission. Patient 2 was also seropositive for *B. henselae*, with a titer of 1:64 on admission. Serial serum specimens obtained from this patient revealed a 6-fold rise in antibody titers 6 days after admission; titers continued to rise to 1:4096 at day 20 after admission. Forty-five days after admission, serum titers had decreased to 1:1024. Serum titers of both patients observed for more than 3 months remained elevated but stable.

**Results From Calcium Metabolism Studies.—** On admission and 6 and 7 days later, both patients had increased levels of serum and urinary calcium, serum phosphate, and serum 1,25(OH)₂D, whereas they had normal values of serum 25-hydroxyvitamin D and urinary cyclic adenosine monophosphate and decreased serum concentrations of intact parathyroid hormone (Table). Neither patient had hypercalcemia-related symptoms at any time. Sixteen days after admission in patient 1 and 20 days after admission in patient 2, these abnormalities were all resolved (Table). No medications were administered that could have affected calcium metabolism.

**Comment**

In these patients a direct correlation was observed between the serum 1,25(OH)₂D levels and both the serum and the 24-hour urinary calcium concentrations. This fact supports the hypothesis that excessive serum 1,25(OH)₂D was responsible for the development of hypercalcemia in both patients. The 3 most frequent causes of human hypercalcemia are primary hyperparathyroidism, malignant neoplasms, and granulomatous diseases. Deregulated production of 1,25(OH)₂D by macrophages that are activated appears to be the pivotal causative factor for the abnormalities of calcium metabolism that may occur in some granulomatous diseases, especially sarcoidosis. Overproduction of 1,25(OH)₂D may cause increased intestinal absorption of calcium, enhanced bone resorption, and resultant hypercalcemia and without hypercalcemia. The extrarenal synthesis of active vitamin D sterols has a central causative role in the hypercalcemic hypercalcemic state associated with sarcoidosis, tuberculosis, disseminated candidiasis, leprosy, silicone-induced granulomatous disease, Wegener granulomatosis, and *Nocardia asteroides* infection, whereas the circulating vitamin D metabolite status of patients with hypercalcemia who have other granuloma-forming diseases, such as coccidioidomycosis, histoplasmosis, berylliosis, and eosinophilic granuloma, has not been carefully studied. To the best of our knowledge, our 2 cases are the first instances of hypercalcemia reported in patients with CSD.

Stellate caseating granulomas, usually with microabscesses formation, are widely thought to be the characteristic distinguishing histopathologic feature of CSD. Of interest, our patients had numerous necrotizing granulomas in their lymph node biopsy samples. The fact that they were monozygotic twins and that the serum 25-hydroxyvitamin D and that 1,25(OH)₂D concentrations were not routinely evaluated in most patients with CSD, one might speculate that abnormalities in calcium metabolism may have been underdiagnosed in other patients with CSD. In fact, the prevalence of hypercalcemia in patients with sarcoidosis is much higher than that of hypercalcemia.

There are now at least 3 major lines of clinical evidence to demonstrate that the extrarenal, macrophage-dependent synthesis of an active vitamin D metabolite in hypercalcemic-hypercalcemic patients with sarcoidosis is not subject to control by those factors that normally regulate renal 1α-hydroxylase. First, hypercalcemic patients have a frankly high or inappropriately elevated serum 1,25(OH)₂D concentration, although their serum intact parathyroid hormone level is suppressed and their serum phosphate concentration is relatively elevated. Second, the serum concentration of 1,25(OH)₂D is exquisitely sensitive to an increase in the availability of substrate. Third, the serum calcium and 1,25(OH)₂D concentrations are positively correlated to indices of disease activity; patients with sarcoidosis who have widespread disease and high angiotensin-converting enzyme activity are more likely to be hypercalcemic or frankly hypercalcemic.

Most of these features can be extrapolated to the present cases of CSD.

Of importance, we decided not to treat hypercalcemia in our patients because it caused no symptoms. Also, no antibiotic therapy was administered. This allowed us to observe the natural evolution of the infectious disease, including both clinical and serologic evolution as well as the natural evolution of calcium metabolism abnormalities.

Our cases provide evidence that CSD, like other granulomatous diseases, can produce hypercalcemia through the unregulated production of the metabolite 1,25(OH)₂D. Furthermore, in view of these 2 cases, we believe that CSD should be added to the list of granuloma-forming diseases that are responsible for 1,25(OH)₂D-mediated hypercalcemia.

**References**

7. Regnery RL, Olson JG, Perkins BA, Bibb W. Serologic response to *Rochalimaea henselae* anti-

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