CALCITRIOL, VITAMIN D_3, AND MULTIPLE SCLEROSIS

September 3, 2014; Dr. C.E. Hayes, Ph.D., Professor of Biochemistry, U. Wisconsin, Madison, WI

I authored a research study examining one calcitriol dose plus supplementary vitamin D_3 as a treatment for a rodent disease used as a multiple sclerosis (MS) model (1). The unmet need for an effective, safe, and inexpensive MS treatment prompted our study. Formal clinical studies have not tested this approach in MS. This research summary provides information to aid translational research efforts. It is not intended and should not be used as medical advice.

Is there a sound scientific rationale for examining calcitriol and vitamin D_3 treatment in MS? In MS, autoimmunity to myelin components coupled with neurodegenerative processes are believed to cause axonal demyelination and impaired axonal transmission. Abundant and persuasive scientific evidence suggests that low vitamin D status *in utero*, childhood, adolescence, and later interacts with genetic risk factors to propell the MS disease process. This evidence is sufficiently strong that many scientists believe it meets the Bradford-Hill criteria for declaring a causal relationship between an environmental factor and a disease (2). This evidence is summarized in chapters (3, 4), reviews (5), and a newsletter ("Vitamin D3 News for Multiple Sclerosis Patients"; available on my departmental web site). Thus, there is a sound scientific rationale for an MS therapeutic approach involving vitamin D_3 and calcitriol.

What is vitamin D_3, how is it related to calcitriol, and how do they work? Vitamin D_3 is a small, inactive molecule that is formed from 7-dehydrocholesterol in skin that is exposed to sunlight. The liver converts vitamin D_3 into 25-hydroxyvitamin D_3 ($25(OH)D_3$), which circulates in the blood as a biologically inactive hormone precurser. Kidney, immune system, central nervous system (CNS), intestinal tract, skin, and probably other tissues convert $25(OH)D_3$ into calcitriol. Calcitriol is a biologically active hormone that works by turning the expression of particular genes up or down, depending on the gene, the type of cell, and the cell's microenvironment. This elegant system enables biological processes like growth, metabolism, reproduction, and immunity to be coordinated according to sunlight's cues.

Does supplementary vitamin D_3 benefit individuals with MS? Remarkable benefits of supplementary vitamin D_3 have been documented in MS and optic neuritis (ON) patients with low vitamin D status. Canadian MS patients who took an average of 14,000 IU/day of vitamin D_3 had fewer relapses and less disability progression than controls over one year (6); 1000 IU = 25 μg. Finnish MS patients who took 20,000 IU/wk of vitamin D_3 had 85% fewer new MRI lesions, and trends toward lower total lesion burden, reduced EDSS, and improved walking scores than controls over one year (7). American MS patients who began taking vitamin D_3 during a 5-year observational study had 33% fewer new MRI lesions for each 10 ng/mL increase in 25(OH)D (8). Iranian ON patients who took 50,000 IU/wk of vitamin D_3 had fewer new brain lesions and a 68% lower risk of developing MS than controls (9). The few studies that did not report benefits involved few subjects, short observation periods, and/or vitamin D_2 rather than vitamin D_3.

What vitamin D_3 supplementation plan is optimal? Ongoing studies aim to address this unanswered question. While we await new information, an important first step is measuring the blood concentration of 25(OH)D as an indicator of vitamin D status. MS relapse hazard declined linearly with rising 25(OH)D levels, regardless of disease therapy, disease duration, EDSS score, season, skin pigmentation, personal UV exposure, number of infections and other potential confounders (10). Relapse rates were lowest in patients whose 25(OH)D levels were 100nmol/L (1nmol/L = 0.4μg/L) or higher, so this value serves as a target until more specific data are forthcoming. Variations in season, latitude, sun exposure, skin pigmentation, and genetics influence 25(OH)D levels (11, 12). Consequently, individuals differ in the vitamin D_3 supplement needed to achieve the target level. Endocrine Society guidelines on vitamin D_3 nutrition (12) can be followed for 2-3 months, and the blood 25(OH)D level re-checked. If it is below the target, a gradual vitamin D_3 dose escalation with intermittent re-checking will identify the daily vitamin D_3 supplement needed. MS patients can safely take up to 14,000 IU/day of vitamin D_3 (6). Vitamin D_2 is less active, so vitamin D_3 is preferred (13).

Does calcitriol benefit individuals with MS? Some MS patients experience disease progression despite supplementary vitamin D_3 prompting the question - would calcitriol benefit them? A pilot study tested daily calcitriol in 15 MS patients (14). All patients had a relapse in the year before enrollment. Calcium intake was limited to 800 mg/day. Oral calcitriol (Rocaltrol, Roche Pharmaceuticals) dosing began at 0.5 μg/day and was increased in 0.5 μg increments over 8 wks to 2.5 μg/day. This dose was maintained for 48 wks. Two patients who did not limit their calcium intake developed hypercalcemia. During the study 27% of patients had a relapse. The mean EDSS score was 2.2 at baseline and study termination. In the ensuing year, the mean EDSS score increased to 3.1 and some patients progressed to EDSS 6. Thus, daily calcitriol treatment appeared to have beneficial effects, but the hypercalcemia risk was considered unacceptable.

Why was the single calcitriol dose protocol tested in rodent studies and what were the results? We sought to retain calcitriol's benefits but limit the hypercalcemia risk. In cancer patients, intermittent calcitriol dosing allowed significant dose escalation without hypercalcemia (15, 16). We reasoned that this approach might be useful for a T cell-mediated demyelinating disease, because calcitriol rapidly stimulated the death of pathogenic autoimmune T cells (17, 18). In rodents, the calcitriol dose increased serum calcitriol for only a few hours, long enough to stimulate CNS-invading autoimmune T cell death, but not long enough to stimulate an increase in serum calcium (1). Combined with supplementary vitamin D_3, the single calcitriol dose induced lasting remissions in 100% of animals, reduced mortality from 27% to 0%, and diminished the cumulative disease score by 48%. Analysis of T cells, spinal cord and optic nerve sections confirmed the clinical data. This protocol was superior to methylprednisolone, interferon-beta, glatiramer acetate, and antibodies to alpha4 integrin for treating rodent immune-mediated demyelinating disease.

What concerns arise in translating the single calcitriol dose protocol from the rodent model disease to MS patients? The first question is whether rodent EAE disease faithfully models the human disease. EAE models the neuroimmunological aspects of MS particularly well (19). However, the drivers of EAE disease are explicitly known, whereas the drivers of MS disease are largely unknown. EAE proved useful in developing interferon-beta,

glatiramer acetate, and antibodies to alpha4 integrin as MS therapies. Other agents have not translated from EAE to MS, lending uncertainty to translational research outcomes. The calcitriol pilot study in MS patients (14) hints that intermittent calcitriol administration may be beneficial in MS patients.

The calcitriol doses to test in MS translational research are unknown. Oncologists performed an informative oral calcitriol safety study in cancer patients (15). Exclusion criteria were pregnancy, history of hypercalcemia, kidney stones, heart failure or heart disease, current digoxin, thiazide, bisphosphonate, or systemic steroid therapy, or use of magnesium-containing antacids, bile resin-binding drugs, or calcium supplements. Calcium intake was limited to 500 mg/day and water intake was increased. They performed a dose escalation study starting with 0.06 μg/kg of calcitriol divided in 4 doses over 4 hrs (using FDA tables for dose conversion between species based on body surface area, our starting dose in rodents, 1 ng/g, would be roughly equivalent to 0.08 μg/kg in humans). Participants who had no grade 3 or higher toxicity were permitted to enroll for a 2-fold higher calcitriol dose the following week. Calcitriol absorption plateaued at 0.48 μg/kg, so there is no rationale for a higher dose. Adverse events were monitored as described (15). All observed toxicities were self-limiting; calcium levels above normal range returned to normal within 2 days without intervention. Calcitriol dose escalation studies in MS patients are urgently needed to ascertain the safety of this calcitriol pulse dose approach.

At least two MS patient populations warrant consideration for translational research. MS patients experiencing breakthrough disease despite receiving therapy would be one population. Glucocorticoid treatment is the standard of care for these patients (20). However, repeated treatment can have serious adverse effects (22), 59% of MS patients are glucocorticoid resistant (23), and glucocorticoid therapy suppresses natural calcitriol biosynthesis (21), highlighting a need for effective alternatives. A randomized controlled trial could compare glucocorticoid standard of care to calcitriol pulse dose treatment. Since significant benefits of supplementary vitamin D_3 have been documented in MS patients, ethical considerations suggest that all study participants should receive a vitamin D_3 supplement (e.g. 2000 IU/day). Benefits expected might be increased frequency and duration of remissions and decreased frequency and severity of adverse events.

Primary progressive MS (PPMS) patients for whom there are no FDA-approved therapies (24) would also warrant consideration for translational research. PPMS is a rare chronic disease with a prevalence less than 1 in 4000. This limits the clinical trial design choices. The crossover trial, wherein each participant serves as his/her own control, is particularly useful for rare chronic diseases (25). Introducing patient choice to escape early increased study power and limited exposure to ineffective treatment in the crossover trial design (25). Crossover studies require that the test agent have a short washout period, which is true of a calcitriol pulse dose. Assessing outcomes in MS research is difficult when performed in the aggregate, due to individual variation in disease course and treatment response. However, repeated functional tests of ambulation, strength, balance, dexterity, visual acuity, fatigue, pain, cognition, and well-being over time might reveal significant individual outcome trends in PPMS in a cost effective manner. Benefits expected might be improvement trends in these parameters while on intermittent calcitriol pulse dosing compared to the trends while on placebo. Soon, we hope to report calcitriol mechanism-based biomarkers to aid in MS translational research.

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