The effect of vitamin D3 on CD34 progenitor cells in vitamin D deficiency rickets

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Vitamin D metabolites have multiple functions not only in calcium homeostasis, but also in hematopoiesis. To detect the effect of vitamin D on hematopoiesis with a surface glycoprotein marker, the proportions of the CD34+ cells were measured in bone marrow, peripheral blood and spleen prior to and after vitamin D3 treatment in an infant with severe rickets, myelofibrosis and myeloid metaplasia. CD34+ cells measured 0.4% in bone marrow, 8.0% in peripheral blood and 8.7% in splenic aspirate. The detection of a high and comparable level of CD34+ cells in both peripheral blood and splenic aspirate on admission and the decline in the level of CD34+ cells (2%) following treatment support that CD34+ cells were from extramedullary hematopoiesis in spleen. The improvement of rickets and hematological findings with treatment at the same time raises the possibility of vitamin D3 acting directly upon the same target or upon different targets at the same time or of the presence of interaction between two targets. Our findings may also show a relation between vitamin D3 and its metabolites to bone marrow stem cells.

Key words: rickets, CD34 cells, infant, hematopoiesis, vitamin D.

Traditionally perceived as a secosteroid hormone involved in calcium homeostasis, vitamin D metabolites might have roles in immunoregulation and hematopoietic differentiation[1]. The effect on bone remodeling and the hematopoietic system was examined in cases with vitamin D deficient rickets[2,3]. It was shown that 1,25 $(OH)_2D3$ inhibited the proliferation of colonogenic blast cells and induced acute myeloblastic leukemia (AML) cell lines to differentiate into macrophage-like cells in vitro[4-6]. In contrast, normal human granulocyte-monocyte committed stem cells are slightly stimulated into their clonal proliferation by 1,25 $(OH)_2D3$[7].

The CD34 is a surface glycophosphoprotein expressed on committed and primitive hematopoietic stem cells[8]. In normal individuals, CD34+ cells comprise approximately 1 to 5% of bone marrow cells and 0.1% of peripheral blood cells[8]. CD34+ cells can thus be examined to detect the changes in hematopoietic stem cells. In a previous study, it was shown that the proportion of D34+ cells in peripheral blood did not change in rachitic patients without myeloid metaplasia[9]. Grande et al.[10] reported that physiological levels of vitamin D promote a differentiation of CD34+ hematopoietic progenitors characterized by the induction of all the monomacrophagic imunophenotypic and morphological markers in a liquid culture model; however, there was no study about changes in the CD34 cells in rachitic patients with myelofibrosis.

Previously, we reported hematopoietic suppression in children with vitamin D deficient rickets and the improvement of the hematologic findings with administration of vitamin D[2,3]. In an infant with rickets, myelofibrosis and myeloid metaplasia, we examined the proportion of the CD34+ cells in bone marrow, peripheral blood and spleen prior to and after vitamin D treatment, to clarify the effect of vitamin D on hematopoiesis with a surface glycoprotein marker, the CD34, in vivo.

Case Report

A five-month-old was referred to Hacettepe Children's Hospital because of diarrhea, anemia and hepatosplenomegaly. His weight was 5000 g (<5th percentile), length 59 cm (<5th

percentile) and head circumference 38.5 cm (<5th percentile). Physical examination revealed pallor, metaphyseal enlargement of wrists, rachitic rosaries, craniotabes, hepatomegaly (5 cm below the right costal margin) and splenomegaly (6 cm below the left costal margin). Laboratory data were as follows: hemoglobin 6.7 g/dl, hemotocrit 23%, reticulocyte 3%, leukocytes $4.7X10^9$/L (41% neutrophils, 3% bands, 5% myelocytes, 2% metamyelocytes, 11% normoblasts, 9% monocytes, 29% lymphocytes), and platelet count $62x10^9$/L, erythrocyte anisopoikilocytosis was noted in the blood smear. Serum calcium was 8 mg/dl, phosphorus 1.5 mg/dl, alkaline phosphatase 437 U/L, total protein 6.4 g/dl, and albumin 3.9 g/dl. Liver and renal function tests were normal. The X-ray findings of wrist were typical for rickets. When examined for hemolytic anemia, results of direct and indirect Coombs' test and cold agglutinins were negative. Fetal hemoglobin was 5%, appropriate for his age. The results of parental hemoglobin electrophoresis were normal. Sickling test was negative. Serum iron level was 120 µg/dl and serum iron binding capacity 250 µg/dl. The levels of serum immunoglobulins were normal. Serologic tests for rubella and toxoplasma were negative.

Bone marrow aspirations which were done three times yielded no bone marrow specimen. Bone marrow was obtained by bone marrow biopsy and myelofibrosis was detected. Splenic aspirates revealed myeloid metaplasia. The CD34 counts were 8.0% in peripheral blood, 0.4% in bone marrow and 8.7% in splenic aspirate.

The child was treated with a single oral dose of vitamin D (600,000 IU). Three months after treatment, the patient's liver and spleen returned to normal size. Hemoglobin level was 11.0 g/dl, leukocyte count $6.3x10^9$/L, platelet count $149x10^9$/L, and CD34 counts 2%. The infant's previously described morphological abnormalities in peripheral blood smear had resolved.

Discussion

In this case the diagnosis of rickets due to vitamin D deficiency was based on a history of inadequate vitamin D intake and clinical findings and absence of any other disease causing these hematological findings and was proven by serum assay, X-ray and prompt reversal after vitamin D treatment.

In our clinic, in cases with vitamin D deficiency rickets, hepatosplenomegaly, anemia, thrombocytopenia, myeloid metaplasia and myelofibrosis have previously been reported. All the clinical and hematological findings, including findings of rickets improved, following vitamin D treatment in our patients[2,3]. In this case it was thought that myelofibrosis in bone marrow caused hematopoietic suppression and enlargement of spleen as a result of extramedullary hematopoiesis. As $CD34^+$ cells decreased in bone marrow, the detection of a high and comparable level of $CD34^+$ cells in both peripheral blood and splenic aspirate strongly suggested that $CD34^+$ cells originated from the spleen. The decline in the level of $CD34^+$ cells (from 8% to 2%) following treatment supports that the origin of $CD34^+$ cells was extramedullary hematopoiesis in the spleen in this patient with severe rickets and myelofibrosis. It was reported that vitamin D has differentiation on multi-lineage or myeloid precursor cells instead of the increase in hematopoietic cells[4-6]. Furthermore, vitamin D3 has been shown to be a very potent inhibitor of $CD34^+$ leukemic blasts while preserving clongenic activity of normal human $CD34^+$ hematopoietic progenitor cells[4,6]. In our rachitic case with myelofibrosis, the level of $CD34^+$ cells in bone marrow was decreased. This data shows the proliferation effect of vitamin D on bone marrow stem cells in addition to the differentiation effect. However, this can be valid for $CD34^+$ cells. In addition, the improvement of rickets and hematological findings with treatment at the same time raises the possibility of vitamin D acting directly upon the same targets or upon different targets at the same time or of the presence of interaction between two targets.

The intiation and maintenance of hematopoiesis is a complex process that depends on the participation of support cells, which generate the micro environmental conditions that ensure the size of the stem cell pool and regulate the differentiation of hematopoietic stem cells into the required mature blood cells[11]. In addition, it is known that osteoblastogenesis is temporarily linked to the initiation of bone marrow hematopoiesis. Osteoblasts are one of the stroma layers in primary bone marrow which regulate the differentiation of osteoclasts. In addition, osteoblast cultures have the potential to produce several hematopoietic

cytokines[11-13]. However, due to a lack of osteoblast specific cell-surface markers, the link between hematopoiesis and osteoblasts has not been easy to define. Recently, Visnjic et al.[14] reported that hematopoietic cell development in the bone marrow was arrested in mice with an induced osteoblast deficiency. In addition, the conversion of HL-60 cells to multinuclear giant cells and to cells involved in bone matrix degradation supports that the osteoclasts are also from hematopoietic stem cell origin and are thought to be derived from immature marrow cells of monocyte/macrophage lineage[5,15]. Thus, in view of the evidence, it is likely that bone tissue and hematopoietic cells might be from the same cell origin.

Morphologic and functional studies have shown that immature mouse myeloid leukemia cells and the human promyelocytic leukemia cell line (HL-60) differentiated to mature macrophage-like cells upon treatment with 1-25 $(OH)_2$ D_3 with the reduced proliferation of these cells[4-6]. Our study is an evidence that vitamin D_3 treatment may have suppressed extramedullary hematopoiesis in vivo in a rachitic case with myelofibrosis, and both, differentiation and proliferation in bone marrow. It is concluded that vitamin D could exert its influence not only by stem cell differentiation but also by proliferation.

REFERENCES

1. Walters MR. Newly identified actions of the vitamin D endocrine system. Endocr Rev 1992; 13: 719-764.
2. Yetgin S, Özsoylu Ş. Myeloid metaplasia in vitamin D deficiency rikets. Scand J Haematol 1982; 28: 180-185.
3. Yetgin S, Özsoylu Ş, Ruacan Ş, Tekinalp G, Sarıalioğlu F. Vitamin D deficiency rickets and myelofibrosis. J Pediatr 1989; 114: 213-217.
4. Koeffler HP, Amatruda T, Ikekawa N, Kobayashi Y, DeLuka HF. Induction of macrophage differentiation of human normal and leukemic myeloid stem cells by 1, 25-dihidroxyvitamin D_3 and its fluorinated analogues. Cancer Res 1984; 44: 5624-5628.
5. Mangelsdorf DJ, Koeffler HP, Donaldson CA, Pike JW, Haussler MR. 1,25 dihidroxy vitamin D_3 induced differentiation in a human promyelocytic leukemia cell line (HL-60): receptor-mediated maturation to macrophage-like cells. J Cell Biol 1984; 98: 391-398.
6. Munker R, Norman AW, Koeffler HP. Vitamin D compounds: effect on clonal proliferation and differentiation of human myeloid cells. J Clin Invest 1986; 78: 424-430.
7. Elstner E, Lee YY, Hashiya M, et al. 1α25-dihydroxy-20-epi-vitamin D_3 an extraordinary potent inhibitor of leukemic cell growth in vitro. Blood 1994; 84: 1960-1967.
8. Krause DS, Fackler MJ, Civin CI, May WS. CD34: structure, biology, clinical utility. Blood 1996; 87: 1-13.
9. Yetgin S, Yalçın SS, Demirçeken F, Teziç T, Tuncer AM. CD34 progenitor cells in children with vitamin D deficiency rickets. Blood 1999; 94 (S1): 136b.
10. Grande A, Montanari M, Tagliafico E, et al. Physiological levels of 1alpha, 25 dihydroxyvitamin D3 induce the monocytic commitment of CD34+ hematopoietic progenitors. J Leukoc Biol 2002; 71: 641-651.
11. Dexter TM. Allen TD, Lajtha LG. Conditions controlling the proliferation of hematopoietic stem cells in vitro. J Cell Physiol 1977; 91: 335-344.
12. Taichman RS, Emerson SG. The role of osteoblasts in the hematopoietic microenvironment. Stem Cells 1998; 16: 7-15.
13. Taichman RS, Reilly MJ, Emerson SG. Human osteoblasts support human hematopoietic progenitor cells in vitro bone marrow cultures. Blood 1996; 87: 518-524.
14. Visnjic D, Kalajzic Z, Rowe D, Katavic V, Lorenzo J, Aguila HL. Hematopoiesis is severely altered in mice with an induced osteoblasts deficiency. Blood 2004; Jan 15 [Epub ahead of print].
15. Hayashi S, Yamane T, Miyamoto A, et al. Commitment and differentiation of stem cells to the osteoclast lineage. Biochem CellBiol 1998; 76: 911-922.