VITAMIN D: A MODULATOR OF CELL PROLIFERATION AND DIFFERENTIATION

H. A. P. Pols,[1] J. C. Birkenhäger,[1] J. A. Foekens[2] and J. P. T. M. van Leeuwen[1]

[1]Department of Internal Medicine III, Erasmus University Medical School and
[2]The Dr Daniel den Hoed Cancer Center, Rotterdam, The Netherlands

Summary—1,25-Dihydroxyvitamin D_3, [1,25$(OH)_2 D_3$], the biologically most active metabolite of vitamin D_3, is involved in the regulation of calcium homeostasis and bone metabolism. Recently, receptors for 1,25$(OH)_2 D_3$ have also been shown in cells and tissues not directly related to calcium homeostasis. Experimental data obtained with leukaemic and cancer cell lines, both *in vitro* and *in vivo*, showed the effects of 1,25$(OH)_2 D_3$ on cell differentiation and proliferation. However, high doses of the sterol have to be used to observe these effects. Additional studies are needed to establish whether 1,25$(OH)_2 D_3$ or suitable analogues have a therapeutic potential in malignant diseases without unacceptable toxicity like the development of hypercalcemia.

INTRODUCTION

Studies of the function and endocrinology of vitamin D over the last 80 years have elucidated its role as an important regulator of calcium and phosphate homeostasis. However, current evidence also suggest that the vitamin D endocrine system is involved in the modulation of a number of fundamental cellular processes not directly related to calcium homeostasis (for reviews see Refs [1–3]). In this brief review the effects of the most active metabolite of vitamin D, 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2 D_3$] on cell differentiation and proliferation will be discussed.

VITAMIN D: METABOLISM AND MODE OF ACTION

The term vitamin D is a misnomer: cholecalciferol is not a vitamin in the generally accepted sense of an essential dietary requirement. It is synthesized photochemically in the skin and adequate amounts are produced with sufficient exposure to sunlight. It is also absorbed from the diet. 1,25$(OH)_2 D_3$, is formed by two subsequent hydroxylations in the liver (25-hydroxylase) and the kidney (1α-hydroxylase) and exerts its effect via a specific receptor [3]. The 1,25$(OH)_2 D_3$ receptor belongs to the superfamily of steroid hormone receptors [4]. 1,25$(OH)_2 D_3$ receptors are not confined to the classical target tissues, which play an important role in calcium homeo-stasis but these receptors are also found in a wide variety of so-called non-classical target tissues (Table 1) [3]. In several of these target tissues 1,25$(OH)_2 D_3$ undergoes an extensive side-chain oxidation at the C24 position [2]. This catabolism of the hormone is induced by 1,25$(OH)_2 D_3$ itself and may have a dual effect: (1) directly by regulation of the cellular concentration of the hormone; and (2) indirectly by its ability to modulate the ligand-dependent up-regulation of the 1,25$(OH)_2 D_3$ receptor [5].

Table 1. Tissue distribution of the 1,25$(OH)_2 D_3$ receptor

Normal mammalian tissues or cell types
Intestine
Kidney
Parathyroid glands
Bone
Skin (epidermal and fibroblasts)
Skeletal muscle (myoblast)
Cardiac muscle
Mammary tissue
Testes
Ovary
Uterus
Placenta
Pancreas
Colon
Parotid gland
Thymus
Circulating lymphocytes (activated)
Circulating monocytes
Malignant tissues or cancer-cell lines
Osteosarcoma
Melanoma
Breast carcinoma
Colon carcinoma
Medullary thyroid carcinoma
Myeloid and lymphocyte leukaemia
Pancreatic adenocarcinoma
Transitional bladder carcinoma
Cervical carcinoma
Fibrosarcoma

According to Reichel *et al.* [3].

EFFECTS ON CELL PROLIFERATION AND DIFFERENTIATION

The identification of $1,25(OH)_2D_3$ receptors in tissues not directly related to mineral homeostasis raises the question whether $1,25(OH)_2D_3$ has other functions. In 1981 Abe *et al.* [6] demonstrated for the first time that $1,25(OH)_2D_3$ induces differentiation of mouse myeloid leukaemia cells into monocytes–macrophages. This original observation was extended to other tissues and cells, especially haematopoietic and cancer cell lines [7, 8]. All these studies indicated that $1,25(OH)_2D_3$ may play a role in the regulation of cell differentiation and/or proliferation.

Haematopoietic cells

Leukaemic cell lines are frequently used models to study the effects of $1,25(OH)_2D_3$ on haematopoietic differentiation. The overall effect of treatment of these cells with $1,25(OH)_2D_3$ is a reduced proliferation and a preferential differentiation along the monocyte–macrophage pathway [8]. It is of interest that these alterations are preceded by modulations in the expression of oncogenes. Reitsma *et al.* [9] showed that $1,25(OH)_2D_3$ reduces c-myc mRNA levels in HL-60 cells within 4 h of exposure to the sterol and that this change precedes the onset of other measured phenotypic changes by at least 8 h. Other investigators showed that this inhibition of c-myc gene expression is accompanied by a decreased expression of the c-myb gene. At the same time a transient activation of the c-fos and a sustained expression of c-fms was observed [10]. Finally, recent evidence suggest that the action of $1,25(OH)_2D_3$ to induce Hl-60 cell differentiation and modulate c-myc transcription requires protein kinase C activity [11]. Whether the changes in expression of oncogenes are directly related to differentiation remains to be elucidated.

$1,25(OH)_2D_3$ not only induces *in vitro* differentiation of leukaemic cell lines but also of normal bone marrow progenitor cells into monocyte–macrophages [12, 13]. Furthermore, $1,25(OH)_2D_3$ seems to be involved in the activation and also the fusion of macrophages to multinucleated giant cells [8]. With respect to mineral homeostasis it is important to emphasize that $1,25(OH)_2D_3$ also seems to play a role in the osteoclastogenesis. Immature bone marrow cells of the monocyte–macrophage lineage are thought to be precursors of the bone-resorbing cells, the osteoclasts. In this way, $1,25(OH)_2D_3$

may regulate skeletal homeostasis, at least in part, by modulating differentiation, fusion and activation of haematopoetic progenitors [8].

The marked effects of $1,25(OH)_2D_3$ on growth and differentiation of leukaemic cells *in vitro* led Homna *et al.* [14] to examine whether *in vivo* administration of $1,25(OH)_2D_3$ to nude mice inoculated with murine myeloid leukaemia (M1) cells would decrease leukaemogenicity. Treatment with $1,25(OH)_2D_3$ or $1\alpha(OH)D_3$ [rapidly converted in the liver to $1,25(OH)_2D_3$] considerably prolongs the survival of the mice compared to vehicle-treated animals.

Studies in humans are scanty. Preliminary data obtained by Cunningham *et al.* [15] suggested antitumour activity in patients with low grade non-Hodgkin lymphomas. In contrast, treatment of patients with myelodysplastic syndrome with $2\,\mu g$ $1,25(OH)_2D_3$ daily did not result in an apparent improvement [16]. One of the major problems for further clinical studies with $1,25(OH)_2D_3$ is hypercalcemia, because supraphysiological doses are needed.

Cancer cells

As shown in Table 1, $1,25(OH)_2D_3$ receptors are present in a wide variety of cancer cell lines and tissues. The initial observation that $1,25(OH)_2D_3$ inhibits cell growth in cancer cells was made in melanoma cells [17], and subsequently similar effects were observed in a still growing number of other cancer cell lines [7]. In our laboratory we have studied the effect on cell growth in the rat osteosarcoma cell UMR-106. As shown in Fig. 1, $1,25(OH)_2D_3$ elicits a time-dependent decrease in the incorporation of [³H]thymidine. However, relatively high
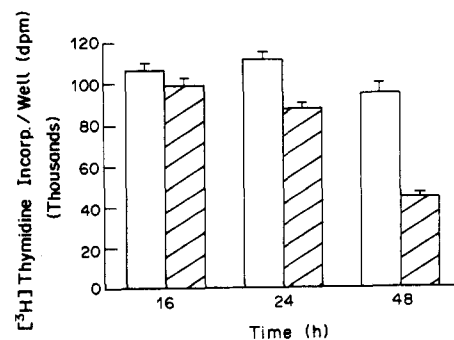

Fig. 1. UMR-106 cells were seeded at a density of 50,000 cells/cm² and cultured in αMEM with 10% FCS. After 24 h the medium was changed to αMEM with 2% charcoal-treated FCS with (▨) or without 10^{-8} M $1,25(OH)_2D_3$ (□). After the times indicated [³H]thymidine incorporation was measured during the final hour of culture, as described by van der Plas *et al.* [30].
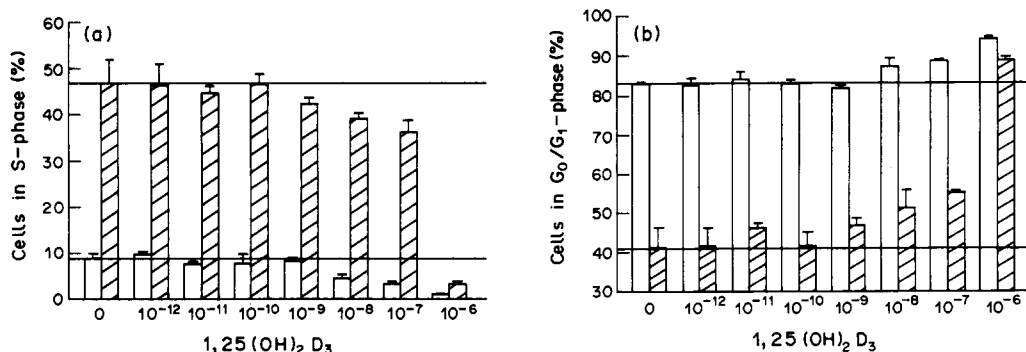
Fig. 2(a,b). MCF-7 cells were seeded at a density of 50,000 cells/cm² and cultured in RPMI-1640 with 10% FCS. After 24 h the medium was changed to RPMI-1640 with 4.5% FCS (□) or 10% FCS (▨). At the same time vehicle or various concentrations of 1,25(OH)₂D₃ were added. Dual-parameter flow propidium iodide uptake in DNA was used to study the effects of 1,25(OH)₂D₃ on cell-cycle kinetics at 24 h of incubation [31].

concentrations of 1,25(OH)₂D₃ (> 10⁻⁹ M) are needed to obtain inhibition of cell growth. Also, in other studies comparable concentrations of 1,25(OH)₂D₃ had to be used to inhibit cell replication *in vitro* [7, 17, 18]. In some cases, inhibition of cell growth is coupled to morphological changes [18–20].

To gain more insight into the effects of 1,25(OH)₂D₃ on proliferation we studied its effect on cell cycle kinetics. For this purpose we used MCF-7 cells, a 1,25(OH)₂D₃ receptor positive human breast cancer cell line. As shown in Fig. 2(a), we observed after 24 h a dose-dependent decrease in the number of cells in the S-phase and a concomitant increase in cell number in the G₀/G₁ phase of the cell cycle [Fig. 2(b)]. During these relatively short incubations no change in the number of cells in the G₂ phase was observed (data not shown). Taken together these results indicate that treatment with 1,25(OH)₂D₃ results in an accumulation of cells in the G₀/G₁ phase of the cell cycle. Comparable results were obtained using the rat osteosarcoma cell line UMR-106 (data not shown). Recently, Eisman *et al.* [21] also showed that in T47D breast carcinoma cells 1,25(OH)₂D₃ inhibited progression through the G₀/G₁ phase. However, in contrast to our observations, they also found a transition delay in G₂ + M. This may be due to differences in experimental conditions, e.g. a longer incubation time (6 days).

It is important to emphasize that for growth reduction by 1,25(OH)₂D₃ supraphysiological concentrations (> 10⁻⁹ M) are needed. Finally, from our own data [Fig. 2(a,b)] the antiproliferative effect of 1,25(OH)₂D₃ in MCF-7 cells seems more potent in rapidly dividing cells cultured in 10% FCS, compared to cells cultured in 4.5% FCS which are growing more slowly [Fig. 2(a,b)]. Whether this indicates an interaction of 1,25(OH)₂D₃ with the response to growth factors present in the medium remains to be elucidated. However, the recent finding of a down-regulation by 1,25(OH)₂D₃ of epidermal growth factor receptors in T47D cells [22] at least suggests that such a mechanism could play a role.

The antiproliferative effect of 1,25(OH)₂D₃ has been confirmed *in vivo*. In high doses the sterol inhibits growth of human malignant melanoma and colonic cancer xenografts in immune suppressed mice [23] and of nitrosomethylurea-induced mammary tumours in rats [24], while administration of 1α(OH)D₃ reduced the number of lung metastases after implantation of Lewis lung carcinoma cells into mice [25]. The fact that 1,25(OH)₂D₃ stimulates fibronectin synthesis in several human cancer cell lines may be related to the possible antimetastatic effect of the hormone [26].

CONCLUSION

From the data currently available it seems clear that 1,25(OH)₂D₃ has a regulatory effect on cell growth and proliferation. However, high doses of the sterol are needed. Therefore, it remains to be established whether 1,25(OH)₂D₃ can produce long-term antitumour effects without unacceptable toxicity, like the development of hypercalcemia. In this light, the recent development of new vitamin D analogues which have potent effects on cell proliferation and differentiation *in vitro* without inducing severe hypercalcemia is of interest [27–29].

REFERENCES

1. Manolagas S. C., Provvedini D. M. and Tsoukas C. D.: Interactions of 1,25-dihydroxyvitamin D₃ and the immune system. *Molec. Cell. Endocr.* **43** (1985) 113–122.
2. Haussler M. R.: Vitamin D receptors: nature and function. *A. Rev. Nutr.* **6** (1986) 527–562.
3. Reichel H., Koeffler H. P. and Norman A. W.: The role of the vitamin D endocrine system in health and disease. *N. Engl. J. Med.* **320** (1989) 980–991.
4. McDonnell D. P., Mangelsdorf D. J., Pike J. W., Haussler M. R. and O'Malley B. W.: Molecular cloning of complementary DNA encoding the avian receptor for vitamin D. *Science* **235** (1987) 1214–1217.
5. Pols H. A. P., Birkenhäger J. C., Schilte J. P. and Visser T. J.: Evidence that the self-induced metabolism of 1,25-dihydroxyvitamin D₃ limits the homologous up-regulation of its receptor in rat osteosarcoma cells. *Biochim. Biophys. Acta* **970** (1988) 122–129.
6. Abe E., Miyaura C., Sakagami H., Takeda M., Konno K., Yamazaki T., Yoshiki S. and Suda T.: Differentiation of mouse myeloid leukemia cells induced by 1,25-dihydroxyvitamin D₃. *Proc. Natn. Acad. Sci. U.S.A.* **78** (1981) 4990–4994.
7. Eisman J. A.: 1,25-Dihydroxyvitamin D₃ receptor and role of 1,25(OH)₂D₃ in human cancer cells. In *Vitamin D Metabolism: Basic and Clinical* (Edited by R. Kumar). Martinus Nijhoff, The Hague (1983) pp. 365–385.
8. Suda T., Miyaura C., Abe E. and Kuroki T.: Modulation of cell differentiation, immune response and tumor promotion by vitamin D compounds. In *Bone and Mineral Research 4* (Edited by W. Peck). Elsevier, Amsterdam (1986) pp. 1–49.
9. Reitsma P. H., Rothberg P. G., Astrin S. M., Trial J., Bar-Shavit Z., Hall A., Teitelbaum S. L. and Kahn A. J.: Regulation of myc gene expression in Hl-60 leukemia cells by a vitamin D metabolite. *Nature* **306** (1983) 492–494.
10. Brevli Z. S., Christakos S. and Studzinski G. P.: Expression of monocyte-specific oncogenes c-fos and c-fms in Hl-60 cells treated with vitamin D₃ analogs correlates with inhibition of DNA synthesis and reduced calmodulin concentration. *Lab. Invest.* **55** (1986) 269–275.
11. Simpson R. U., Hsu T., Wendt M. D. and Taylor J. M.: 1,25-Dihydroxyvitamin D₃ regulation of c-myc proto-oncogene transcription. Possible involvement of protein kinase C. *J. Biol. Chem.* **264** (1989) 19710–19715.
12. Miyaura C., Abe E., Kuroki M., Nishii Y. and Suda T.: 1α,25-Dihydroxyvitamin D₃ suppresses proliferation of murine granulocyte–macrophage progenitor cells (CFU-C). *Biochem. Biophys. Res. Commun.* **108** (1982) 1728–1733.
13. McCarthy D. M., San Miguel J. F., Freake H. C., Green P. M., Zola H., Catovsky D. and Goldman J. M.: 1,25-Dihydroxyvitamin D₃ inhibits proliferation of human promyelocytic leukemia (HL60) cells and induces monocyte–macrophage differentiation in HL60 and normal human bone marrow cells. *Leukemia Res.* **7** (1983) 51–55.
14. Homna Y., Hozumi M., Abe E., Konno K., Fukushima M., Hata S., Nishii Y., DeLuca H. F. and Suda T.: 1α,25-Dihydroxyvitamin D₃ prolong survival time of mice inoculated with myeloid leukemia cells. *Proc. Natn. Acad. Sci. U.S.A.* **80** (1983) 201–204.
15. Cunningham D., Gilchrist N. L., Cowan R. A., Forrest G. J., McArdle C. S. and Soukop M.: Alfacalcidol as a modulator of growth of low grade non-Hodgkin's lymphomas. *Br. Med. J.* **291** (1985) 1153–1155.
16. Koeffler H. P., Hirji K. and Itri L.: 1,25 Dihydroxyvitamin D₃: *in vivo* and *in vitro* effects on human pre-leukemic and leukemic myeloid stem cells. *Cancer Treat. Rep.* **69** (1985) 1399–1407.

17. Colston K., Colston M. J. and Feldman D.: 1α,25-Dihydroxyvitamin D₃ inhibits the clonogenic growth of transformed cells via its receptor. *Endocrinology* **108** (1981) 1083–1086.
18. Frampton R. J., Omond S. A. and Eisman J. A.: Inhibition of human cancer cell growth by 1,25-dihydroxyvitamin D₃ metabolites. *Cancer Res.* **43** (1983) 4443–4447.
19. Dokoh S., Donaldson C. A. and Haussler M. R.: Influence of 1,25-dihydroxyvitamin D₃ on cultured osteogenic sarcoma cells: correlation with the 1,25-dihydroxyvitamin D₃ receptor. *Cancer Res.* **44** (1984) 2103–2109.
20. Gross M., Bollman Kost S., Ennis B., Stumpf W. and Kumar R. Effect of 1,25-dihydroxyvitamin D₃ on mouse mammary tumor (GR) cells: evidence for receptors, cellular uptake, inhibition of growth and alteration in morphology at physiological concentrations of hormone. *J. Bone Miner. Res.* **1** (1986) 457–467.
21. Eisman J. A., Sutherland R. L., McMenemy M. L., Fragonas J. C., Musgrove E. A. and Pang G. Y. N.: Effects of 1,25-dihydroxyvitamin D₃ on cell-cycle kinetics of T47D human breast cancer cells. *J. Cell. Physiol.* **138** (1989) 611–616.
22. Koga M., Eisman J. A. and Sutherland R. L.: Regulation of epidermal growth factor receptor levels by 1,25-dihydroxyvitamin D₃ in human breast cancer cells. *Cancer Res.* **48** (1988) 2734–2739.
23. Eisman J. A., Barkla D. H. and Tutton P. J.: Suppression of *in vivo* growth of human cancer solid tumor xenografts by 1,25-dihydroxyvitamin D₃. *Cancer Res.* **47** (1987) 21–25.
24. Colston K. W., Berger U. and Coombes R. C.: Possible role for vitamin D in controlling breast cancer cell proliferation. *Lancet* (1989) 188–191.
25. Sato T., Takusagawa K., Asoo N. and Konno K.: Antitumor effect of 1α,25-dihydroxyvitamin D₃. *Tohuku J. exp Med.* **138** (1982) 445–446.
26. Franceschi R. T., Linson C. J., Peter C. J. and Romano P. R.: Regulation of cellular adhesion and fibronectin synthesis by 1α,25-dihydroxyvitamin D₃. *J. Biol. Chem.* **262** (1987) 4165–4171.
27. Abe J., Morikawa M. and Miyammoto K.: Synthetic analogues of vitamin D₃ with an oxygen atom in the side chain skeleton: a trial of development of vitamin D compounds which exhibit potent differentiation-inducing activity without inducing hypercalcemia. *FEBS Lett.* **226** (1987) 58–62.
28. Binderup L. and Bramm E.: Effects of a novel vitamin D analogue MC-903 on cell proliferation and differentiation *in vitro* and on calcium metabolism *in vivo*. *Biochem. Pharmac.* **37** (1988) 889–895.
29. Koizumi T., Nakao Y., Ishizuka S., Oshida J., Hara N., Ikekawa N. and Fijita T.: Novel vitamin D₃ derivatives, 26-homo-Δ²²-dehydro-1α,25(S)-dihydroxyvitamin D₃ and 26-homo-Δ²²-dehydro-1α,25(R)-dihydroxyvitamin D₃: preferential activity in c-myc mRNA production and in induction of phenotypic differentiation of HL-60 cells. *Archs Biochem. Biophys.* **276** (1990) 310–316.
30. Plas A. van der, Feyen J. H. M. and Nijweide P. J.: Direct effect of parathyroid hormone on the proliferation of osteoblast-like cells: a possible involvement of cyclic AMP. *Biochem. Biophys. Res. Commun.* **129** (1985) 918–925.
31. Bontebal M., Sieuwert A. M., Klijn J. G. M., Peters H. A., Krijnen H. L. J. M., Sonneveld P. and Foekens J. A.: Effect of hormonal manipulation and doxorubicin administration on cell cycle kinetics of human breast cancer cells. *Br. J. Cancer* **60** (1989) 688–692.