VITAMIN D: A MODULATOR OF CELL PROLIFERATION AND DIFFERENTIATION

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Summary—1,25-Dihydroxyvitamin D3, [1,25(OH)2D3], the biologically most active metabolite of vitamin D3, is involved in the regulation of calcium homeostasis and bone metabolism. Recently, receptors for 1,25(OH)2D3 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)2D3 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)2D3 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 D3 [1,25(OH)2D3] 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)2D3, 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)2D3 receptor belongs to the superfamily of steroid hormone receptors [4]. 1,25(OH)2D3 receptors are not confined to the classical target tissues, which play an important role in calcium homeostasis 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)2D3 undergoes an extensive side-chain oxidation at the C24 position [2]. This catabolism of the hormone is induced by 1,25(OH)2D3 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)2D3 receptor [5].

Table 1. Tissue distribution of the 1,25(OH)2D3 receptor

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

According to Reichel et al. [3].
EFFECTS ON CELL PROLIFERATION AND DIFFERENTIATION

The identification of 1,25(OH)₂D₃ receptors in tissues not directly related to mineral homeostasis raises the question whether 1,25(OH)₂D₃ has other functions. In 1981 Abe et al. [6] demonstrated for the first time that 1,25(OH)₂D₃ 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)₂D₃ 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)₂D₃ on haematopoietic differentiation. The overall effect of treatment of these cells with 1,25(OH)₂D₃ 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)₂D₃ 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)₂D₃ 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)₂D₃ 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)₂D₃ 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)₂D₃ 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)₂D₃ may regulate skeletal homeostasis, at least in part, by modulating differentiation, fusion and activation of haematopoietic progenitors [8].

The marked effects of 1,25(OH)₂D₃ on growth and differentiation of leukaemic cells in vitro led Homna et al. [14] to examine whether in vivo administration of 1,25(OH)₂D₃ to nude mice inoculated with murine myeloid leukaemia (M1) cells would decrease leukaemogenicity. Treatment with 1,25(OH)₂D₃ or 1α(OH)D₃ [rapidly converted in the liver to 1,25(OH)₂D₃] 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 μg 1,25(OH)₂D₃ daily did not result in an apparent improvement [16]. One of the major problems for further clinical studies with 1,25(OH)₂D₃ is hypercalcemia, because supraphysiological doses are needed.

Cancer cells

As shown in Table 1, 1,25(OH)₂D₃ receptors are present in a wide variety of cancer cell lines and tissues. The initial observation that 1,25(OH)₂D₃ 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)₂D₃ elicits a time-dependent decrease in the incorporation of [³H]thymidine. However, relatively high
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


