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VITAMIN D ANALOGUES: NEW REGULATORS OF CANCER CELL GROWTH AND DIFFERENTIATION

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Abstract: 1α,25-dihydroxyvitamin D_3 exerts antitumour effects in vitro and in vivo, but its potential therapeutic value is limited by its strong calcemic effect. New vitamin D analogues with a more favourable therapeutic profile are therefore under development.

In the past 10 years the principle of differentiation therapy in the prevention and treatment of cancer has received an increasing amount of interest. Differentiation therapy is based on the observation that many cancer cells are arrested at an early, immature stage of development and that a number of chemical entities are able to stimulate these cells to differentiate into their mature forms, whereupon they stop proliferating. The best known examples of this type of compounds belong to the vitamin A derivatives, the retinoids. Recently, however, vitamin D metabolites and analogues have shown promise as a new class of regulators of cancer cell growth and differentiation in vitro and in vivo.

Rationale for the use of vitamin D metabolites and analogues in cancer therapy

Vitamin D_3 is hydroxylated in the liver and in the kidneys to its physiologically active form 1α,25-dihydroxyvitamin D_3 ($1\alpha,25(OH)_2D_3$), which plays a major role in calcium homeostasis and bone mineralization[1].

The effects of $1\alpha,25(OH)_2D_3$ are mediated via specific intracellular receptors found in target organs such as bone, intestine and kidneys. In recent years, receptors for $1\alpha,25(OH)_2D_3$ have also been found in many cells and tissues not traditionally involved in the regulation of calcium metabolism (e.g. skin cells, muscle cells, hematopoietic cells) and also in many cancer cells (freshly isolated or established cell lines)[2]. The receptor for $1\alpha,25(OH)_2D_3$ belongs to the superfamily of steroid receptors, comprising receptors for glucocorticosteroids, estrogen, thyroxine and retinoic acid.

After binding to $1\alpha,25(OH)_2D_3$, the receptor complex functions as a transcription factor, binds to vitamin D responsive elements on the genome and regulates the expression of a number of genes involved in calcium homeostasis or in the control of cell growth and

differentiation, depending on the target cells[3]. The growth of breast cancer cells, colon cancer cells, prostate carcinoma cells, melanoma cells, leukemic cells and many others has been shown to be inhibited in cell culture after addition of $1\alpha,25(OH)_2D_3$ in nM concentrations. No cytotoxic effects are seen, but many of the cells acquire the characteristics of a more differentiated phenotype[4]. In some cell types terminal differentiation is achieved.
In tumour-bearing animals, $1\alpha,25(OH)_2D_3$ or its synthetic analogue 1α-hydroxyvitamin D_3 ($1\alpha(OH)D_3$), which undergoes biotransformation in the liver to $1\alpha,25(OH)_2D_3$, have been shown to suppress tumour growth, inhibit metastasis and prolong survival[5,6,7]. Preliminary clinical studies suggest that oral administration of $1\alpha,25(OH)_2D_3$ or $1\alpha(OH)D_3$ may be of benefit in myelofibrosis, myelodysplastic syndromes and non-Hodgkin's lymphomas[8,9,10].

The clinical use of these compounds is however limited by their strong effects on intestinal calcium uptake. Administration of more than a few μg per day leads to hypercalcemia, with the associated risk of inducing soft tissue calcifications. It has therefore been an obvious goal to synthesize analogues of $1\alpha,25(OH)_2D_3$ in the hope to try to eliminate or reduce the calcemic effects, while retaining or increasing the activity on the cell regulatory processes.

Development of new vitamin D analogues

Leo Pharmaceuticals have been engaged in vitamin D research for many years, first with the synthesis of $1\alpha(OH)D_3$ for use in bone disorders in patients with renal failure, and later with the search for new analogues suitable for the treatment of hyperproliferative diseases. An interesting candidate, calcipotriol (MC 903), was found in 1985. This side chain analogue of $1\alpha,25(OH)_2D_3$ was found to be a potent inducer of cell differentiation and inhibitor of cell proliferation in vitro in human histiocytic leukemia cells[11]. Its activity was comparable to that observed with $1\alpha,25(OH)_2D_3$. In addition, calcipotriol was 100-200 times less active than $1\alpha,25(OH)_2D_3$ in causing calcemic side effects, after administration to rats. At the earliest stage of its development, calcipotriol was considered as a promising agent for systemic treatment of cancer. It was, however, rapidly recognized that the low calcemic effect in vivo was mainly related to a rapid rate of metabolic clearance. As a consequence of these findings, calcipotriol was considered more suitable for topical than for systemic use. Today, calcipotriol has been developed as an effective and safe therapy for topical treatment of psoriasis[12]. In psoriasis, calcipotriol normalizes the epithelial hyperproliferation and the incomplete terminal cell differentiation. In the field of cancer, calcipotriol has been

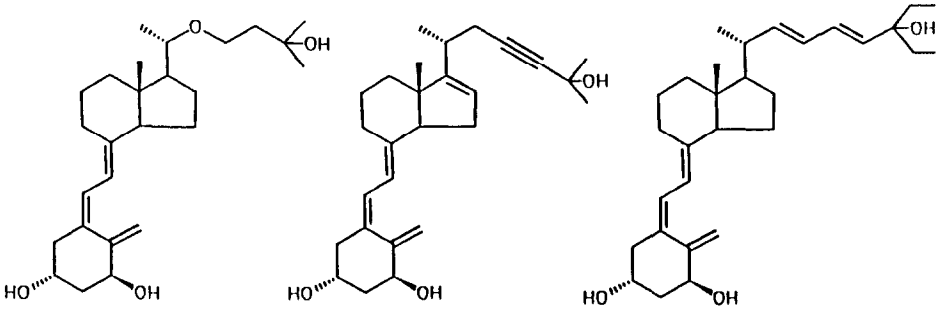
shown to inhibit the growth of cutaneous metastatic breast cancer in a subgroup of patients with tumours possessing receptors for $1\alpha,25(OH)_2D_3$[13].

The next goal has been to develop vitamin D analogues suitable for systemic use in patients with hyperproliferative diseases. So far, only a few vitamin D analogues have been described:

- 22-oxa-$1\alpha,25(OH)_2D_3$ (Chugai Pharmaceuticals), which is able to delay the growth of implanted breast tumour cells in athymic mice[14]
- 16-ene-23-yne-$1\alpha,25(OH)_2D_3$ (Hoffmann-La Roche), which has been shown to prolong survival of mice injected wih leukemia cells[15]
- EB 1089 (Leo Pharmaceuticals), which is able to inhibit the growth of rat mammary tumours induced by the carcinogen nitrosomethylurea[16,17].

These three compounds all inhibit the proliferation of various cancer cell lines in vitro more potently than $1\alpha,25(OH)_2D_3$. They induce differentiation of immature leukemic cells along the monocyte-macrophage pathway and they have reduced calcemic effects in vivo, compared to $1\alpha,25(OH)_2D_3$.

Chemical structures of vitamin D analogues with antitumour effects:



22-oxa-
$1\alpha,25(OH)_2D_3$

16-ene-23-yne-
$1\alpha,25(OH)_2D_3$

EB 1089

Biological effects of EB 1089 in vitro and in vivo

In vitro, EB 1089 has been shown to inhibit proliferation of U 937 human histiocytic lymphoma cells[18]. EB 1089 was approximately 100 times more potent than $1\alpha,25(OH)_2D_3$ (IC_{50} = 2.5 x 10^{-10}M for EB 1089, versus 1.7 x 10^{-8}M for $1\alpha,25(OH)_2D_3$). Differentiation of U 937 cells along the monocyte-macrophage pathway was induced by EB 1089 at 3.0 x 10^{-11}M and by $1\alpha,25(OH)_2D_3$ at 2.0 x 10^{-9}M. Human breast cancer cells (MCF-7) have also been shown to be responsive to growth inhibition by EB 1089 in vitro, with EB 1089 being approximately 50 times more potent than $1\alpha,25(OH)_2D_3$[17,19]. The effects of EB 1089 on regulation of growth and differentiation of MCF-7 cells have further been studied at the level of expression of the c-myc and c-fos protooncogenes. EB 1089 decreased the expression of c-myc mRNA and transiently incresead c-fos expression, being approximately 50 times more potent than $1\alpha,25(OH)_2D_3$[19].

In vivo, EB 1089 has been tested for antitumour effects in rats with mammary tumours induced by nitrosomethylurea[16,17]. Oral treatment with EB 1089 at 0.5 μg/kg/day for 4 weeks resulted in significant inhibition of tumour growth, in the absence of changes in serum calcium levels. The same dose of $1\alpha,25(OH)_2D_3$ had no effect on tumour growth, but caused hypercalcemia. At 2.5 μg/kg/day EB 1089 tumours regressed by more than 80%. This dosage, however, induced hypercalcemia. In rats bearing the Leydig cell tumour H-500, EB 1089 has been shown to prolong survival time and to reduce levels of serum PTH-related peptides, when administered at non-calcemic dosages[20]. Experiments in non-tumour bearing animals have shown that EB 1089 is approximately 50% less calcemic than $1\alpha,25-(OH)_2D_3$, both after oral and intraperitoneal administration[19].

Conclusion

In view of the widespread distribution of receptors for $1\alpha,25(OH)_2D_3$ in cancer cells of both epithelial and myelopoietic origin and of the ability of $1\alpha,25(OH)_2D_3$ and its new analogues to regulate growth and differentiation of these cells, the rationale for their use in cancer therapy seems well established. Clinical trials will establish whether analogues with reduced calcemic effects will be useful, either as monotherapy or, more likely, in combination with established treatment modalities.

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