Vitamin D analogues: from molecule to clinical application

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During the last 70 years our understanding of the significance of vitamin D as an important regulator of calcium and phosphate homeostasis has greatly increased. It is now well established that vitamin D is metabolized first in the liver to 25-hydroxyvitamin D$_3$ and then in the kidney to the active metabolite 1,25-dihydroxyvitamin D$_3$ (1,25-(OH)$_2$D$_3$). The seco-steroid hormone 1,25-(OH)$_2$D$_3$ exerts its principal biological activities through specific intracellular receptors, which are nuclear transcription factors belonging to the steroid receptor superfamily (Haussler, 1986; Reichel et al., 1989; Pols et al., 1990; Bikle, 1992; Walters, 1992). Moreover, the antiproliferative effect of 1,25-(OH)$_2$D$_3$ has been confirmed in vivo. The sterol considerably prolongs survival of nude mice inoculated with murine myeloid leukaemia cells (M1-cells) (Homna et al., 1983), while it also inhibits growth of human malignant melanoma, colonic cancer cell and breast cancer cell xenografts in immune suppressed mice (Eisman et al., 1987; Colston et al., 1989). Administration of 1α-hydroxyvitamin D$_3$ also reduces the number of lung metastases after implantation of Lewis lung carcinoma cells into mice (Sato et al., 1982).

The exciting new properties of 1,25-(OH)$_2$D$_3$ in a number of its non-classic target tissues underlie current efforts to explore new clinical indications for the use of 1,25-(OH)$_2$D$_3$ (Bikle, 1992). Clinical studies in patients with psoriasis have already shown favourable effects of especially topically administered 1,25-(OH)$_2$D$_3$ (Holick, 1989; Bikle & Pillai, 1993). Of course there is also significant interest in the therapeutically potential of 1,25-(OH)$_2$D$_3$ for treatment of malignancies. However, for the antiproliferative (anti-tumour) effect supraphysiological doses of systemically administered 1,25-(OH)$_2$D$_3$ are needed. Thus doses optimal in terms of preventing tumour growth, will inevitably result in complications, like hypercalcaemia and hypercalciuria. These severe complications have prompted the development of vitamin D analogues in an attempt to separate the calcaemic activity from the antiproliferative and cell differentiation activity.

Comparison of the mechanisms of action of vitamin D and its analogues

Most of the analogues currently available are based on extensive modifications of the side-chain of vitamin D (Calverley & Jones, 1992). The selection of analogues for future clinical research has in particular been focused on their ability to decrease proliferation and enhance differentiation of malignant or normal cells in vitro without having apparent calcaemic effects. Nevertheless, attempts have also been undertaken to develop analogues for the more classic indications. For instance, to treat osteoporosis we need analogues which stimulate bone formation, but do not affect bone resorption (Nishii et al., 1993).
For the development and understanding of the action of vitamin D analogues it is important to define the mechanisms involved in the biological action of vitamin D, because the interplay between these mechanisms will determine the ultimate biological responses. Therefore, this review will be focused on the relevance of (1) metabolism and vitamin D binding proteins, (2) receptor binding and (3) aspects which have come forward more recently, namely non-classic steroid hormone messenger systems such as calcium and protein kinase C. It is not our purpose to discuss in detail individual characteristics of the various analogues.

**Metabolism and vitamin D binding proteins**

Tissue and plasma levels of 1,25-(OH)2D3 are dependent upon both synthesis and catabolism. In the kidney 1,25-(OH)2D3 is a negative regulator of its own synthesis. Therefore, a possible site of action for vitamin D analogues is inhibition of the endogenous synthesis of 1,25-(OH)2D3. For the analogue 22-oxacalcitriol (OCT) such an inhibition of 1,25-(OH)2D3 formation in vivo has been observed (Dusso et al., 1992). However, it remains to be established whether such a mechanism is related to the low calcaemic activity of OCT or other vitamin D analogues.

In kidney and intestine, and in most other target tissues, 1,25-(OH)2D3 induces the enzyme 24-hydroxylase. This enzyme initiates a catabolic cascade for the side-chain oxidation, cleavage and ultimate metabolic elimination of 1,25-(OH)2D3 and its precursor 25-(OH)D3 (Kumar, 1986; Haussler, 1986). This so-called C24-oxidation pathway is regarded as a mechanism whereby 1,25-(OH)2D3 can regulate/limit its own biological action in cells. It is conceivable that vitamin D analogues can interact with this local regulatory mechanism in several ways. The ability of analogues to induce the enzyme 24-hydroxylase could be different or even absent (Pols et al., 1991), and modifications in the side-chain could reduce susceptibility to side-chain oxidation.

C24-oxidation contributes only 35–40% to the catabolism of 1,25-(OH)2D3; the remainder consists of other side-chain oxidations and biliary excretion of polar metabolites. In contrast to the C24-oxidation pathway these processes are regulated neither by 1,25-(OH)2D3 nor by calcium or phosphate (Kumar, 1986). Nevertheless these metabolic pathways may also interact with the regulation of the eventual response of vitamin D analogues. It is even possible that in contrast to the metabolism of 1,25-(OH)2D3, the metabolism of analogues results in the accumulation of active metabolites in target cells, as has been reported for 26,27-hexafluoro-1,25-(OH)2D3 (Nagata et al., 1988). Whether this is applicable only to fluorinated analogues, which have an altered side-chain metabolism, remains to be elucidated.

An aspect directly related to 1,25-(OH)2D3 catabolism, and thereby its action, is binding to transport proteins in blood. Vitamin D binding protein (DBP) is characterized by a high affinity and high capacity for vitamin D3 and its metabolites. Besides binding to DBP vitamin D3 metabolites can also bind to albumin and low-density lipoproteins (both low affinity, high capacity) (Haddad, 1987). Pharmacological studies showed that for several analogues a low affinity to DBP seems to be related to a shorter half-life (Kissmeyer & Binderup, 1991; Bouillon et al., 1991). In addition low binding to DBP may decrease the immediate availability of the analogues to target tissues (Bouillon et al., 1991; Norman et al., 1991). These observations are supported by the observation that reduced binding to DBP is mostly correlated with diminished in-vivo activity (Binderup & Bramm, 1988; Bouillon et al., 1991). Also, data obtained with analogues with increased binding to DBP demonstrate a decreased in-vivo activity in rachitic chick (Bouillon et al., 1992). However, in this latter situation not only reduced bioavailability, but also reduced affinity for the VDR has to be taken into account.

The fact that the analogue OCT still has profound effects in vivo, despite its low affinity to DBP and its rapid clearance from the circulation, may be related to its higher affinity for lipoproteins compared to various vitamin D3 metabolites (Kobayashi et al., 1991; Abe et al., 1989; 1991). It is thought that OCT is transported to target tissues bound to lipoproteins and then incorporated into cells via lipoprotein-membrane receptors. In general, this mechanism indicates that, dependent on the presence on target cells of membrane receptors for transporters of vitamin D3 and analogues, the compounds may be delivered in a selective manner.

**Receptor binding**

In the classical model (see also below) 1,25-(OH)2D3 binds to a specific cytosolic/nuclear receptor which subsequently binds to DNA and initiates transcription of various genes resulting in an altered cellular activity. The VDR is a member of the steroid hormone receptor family which have a characteristic structure with distinct ligand and DNA binding domains separated by a hinge-region. Characteristic of the DNA binding domain are the zinc-fingers in which the so-called P-box and D-box are responsible for recognition of DNA sequences and receptor dimerization (O'Malley, 1990; Carson-Jurica et al., 1990). In contrast to the closely related thyroid hormone and retinoic acid hormone receptors only one type of VDR has been identified. Therefore, one can argue that it is unlikely that vitamin D analogues exert tissue
specific actions via tissue specific receptors. However, after receptor binding several processes take place which can be subject to different activation by analogues. Firstly, in response to 1,25-(OH)₂D₃ binding, phosphorylation of the VDR on specific sites appears to be crucial for activation of gene transcription (Pike & Sleator, 1985; Brown & Deluca, 1990; Hsieh et al., 1991; Jones et al., 1991; Orti et al., 1992; Jurutka et al., 1993; Darwich et al., 1993). Secondly, it has been shown that interaction with a nuclear factor (heterodimerization), recently identified as the retinoic X receptor (RXR), is necessary for the binding of the 1,25-(OH)₂D₃-VDR complex to DNA (Liao et al., 1990; Ozono et al., 1991; Ross et al., 1992). Although it remains to be established whether altered VDR phosphorylation and heterodimerization play a role in the action of vitamin D analogues, results with the analogue 26,27-hexafluoro-1,25-(OH)₂D₃, showing a higher binding affinity of the VDR-ligand complex for DNA, at least do not exclude the involvement of these mechanisms (Inabe et al., 1989).

Non-classic steroid hormone mechanisms

Over the last five years responses to 1,25-(OH)₂D₃ have been observed which were previously exclusively associated with the action of peptide hormones and not with the action of steroid hormones. Both 1,25-(OH)₂D₃-induced, but actinomyein D-insensitive, rapid changes in intestinal calcium transport (termed transcalcitachia) (Nemere & Norman, 1987) and rapid changes in intracellular calcium levels in osteoblasts, muscle cells, hepatocytes, keratinocytes, and parathyroid cells (Baran & Milne, 1986; De Boland & Boland, 1987; Lieberherr, 1987; MacLaughlin et al., 1987; Oshima et al., 1987; Sugimoto et al., 1988; Caffrey & Farach-Carson, 1989) in response to the seco-steroid indicate a genomic-independent action. Also, rapid effects of 1,25-(OH)₂D₃ on cellular cyclic GMP levels (Barsony & Marx, 1988) and membrane lipid metabolism in a variety of cells have been reported (Matsumoto et al., 1981; Baran et al., 1990; Baran & Kelly, 1988; Okazaki et al., 1989; Lieberherr et al., 1989; Civitelli et al., 1990). This latter observation might be related to changes in activity of the intracellular signal system protein kinase C (Martelli et al., 1987; Obeid et al., 1990; Simboli-Campbell et al., 1992). Whether these apparently non-genomic effects of 1,25-(OH)₂D₃ are exerted via the classic VDR or via a new membrane-associated receptor remains to be established. However, recent findings in osteoblast-like cells with different vitamin D analogues demonstrate dissociations between VDR binding and stimulation of calcium influx (Farach-Carson et al., 1991). These observations together with data showing a rapid 1,25-(OH)₂D₃-induced increase in intracellular calcium in rat osteosarcoma cells lacking the classical VDR (Baran et al., 1991) support the existence of a distinct plasma-membrane receptor. Studies to isolate and characterize a possible vitamin D membrane receptor are in progress. Nevertheless the functional involvement of calcium and protein kinase C in various biological responses to 1,25-(OH)₂D₃ has already been demonstrated in HL-60 cells and bone (Simpson et al., 1989; Van Leeuwen et al., 1990; 1992) and support the importance of both genomic and non-genomic mechanisms. Therefore, the difference in the ability of the various analogues to activate both genomic and non-genomic mechanisms may contribute to their selective actions.

Clinical applications

The characteristics of the relatively few analogues tested in vivo give some indications of their potential clinical applications. In Table 1, in-vivo studies with several vitamin D analogues are summarized, with the exception of studies which examined only effects on serum and urinary calcium.

The ability of 1,25-(OH)₂D₃ to inhibit PTH synthesis and/or secretion has led to its use in patients with secondary hyperparathyroidism due to chronic renal failure. However, in these patients oral 1,25-(OH)₂D₃ frequently induces hypercalcaemia. Ten years ago Slatopolsky et al. (1984) observed that the intravenous administration of 1,25-(OH)₂D₃ reduced PTH levels more effectively and with considerably less increment of serum calcium than did oral therapy. The recently developed analogue OCT has similar effects to 1,25-(OH)₂D₃ on PTH synthesis and release, but is without a calcaemic effect. Therefore, OCT may provide a useful treatment of secondary hyperparathyroidism (reviewed by Slatopolsky et al., 1992). Consequently, it is tempting to speculate that noncalcaemic analogues can be used in the treatment of primary hyperparathyroidism. At present the use of analogues for modulation of the secretion of other hormones like insulin seems to be more distant (Bikle, 1992).

The synthesis of vitamin D analogues that, at much lower concentrations than 1,25-(OH)₂D₃, are able to inhibit T-lymphocyte activation, may give rise to a new class of immunosuppressive compounds (reviewed by Binderup, 1992). In this respect the analogue, KH 1060, has been shown to be several orders of magnitude more potent than cyclosporin A (Lillevang et al., 1992). In addition, preliminary in vivo experiments indicate that i.p. administered low doses of KH 1060 significantly prolong mouse skin allograft survival (Chiocchia et al., 1991). These findings, together with in vivo studies showing that 1,25-(OH)₂D₃ can modulate the course of autoimmune disorders such as thyroiditis, encephalomyelitis, rheumatoid arthritis and systemic lupus...
The use of vitamin D analogues as antiproliferative compounds is also surrounded with an air of optimism. After the observation of Morimoto and Kumahara (1985), who showed that treatment of an osteoporotic woman with 1α-(OH)D₃ resulted in clearing of her psoriasis, other clinical studies indicate that topical treatment with 1,25-(OH)₂D₃ improves psoriasis (Holick, 1989). Topical application of the analogue Calcipotriol (MC903) in patients with psoriasis resulted in clearing of their skin lesions in over 80% of the patients (Kragballe et al., 1991). No changes in serum calcium levels were observed. Also, topical application of Calcipotriol on cutaneous deposits did prove effective in some patients with advanced breast cancer (Bower et al., 1991). The low calcemic response to Calcipotriol after systemic administration has been related to its poor affinity to DBP and (consequently) its rapid clearance from the circulation (Bouillon et al., 1991; Kissmeyer & Binderup, 1991). Based on these observations one could argue that the usefulness of Calcipotriol hinges simply on its mode of delivery, enabling it to reach its target tissues, but without exerting calcemic effects. However, despite its low binding to DBP, experiments with the analogue 1,25-(OH)₂-16-ene-

Table 1 In-vivo effects of vitamin D analogues

<table>
<thead>
<tr>
<th>Analogue</th>
<th>Effect</th>
<th>Dose</th>
<th>Administration†</th>
<th>Species</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>OCT</td>
<td>Increased primary immune response</td>
<td>0.2–200 ng/kg BW</td>
<td>Oral</td>
<td>Mouse</td>
<td>Abe et al. (1989)</td>
</tr>
<tr>
<td>OCT</td>
<td>Inhibition of PTH synthesis</td>
<td>130 ng/kg BW</td>
<td>i.p. Injection</td>
<td>Rat</td>
<td>Brown et al. (1989)</td>
</tr>
<tr>
<td>OCT</td>
<td>Beneficial effect on autoimmune disorder</td>
<td>2 or 100 ng/kg BW</td>
<td>Oral</td>
<td>Mouse</td>
<td>Abe et al. (1990)</td>
</tr>
<tr>
<td>OCT</td>
<td>Inhibition of embryonic angiogenesis</td>
<td>1 ng/egg</td>
<td>Oral implantation</td>
<td>Chicken</td>
<td>Oikawa et al. (1990)</td>
</tr>
<tr>
<td>OCT</td>
<td>Inhibition of breast cancer growth</td>
<td>10000 ng/kg BW</td>
<td>Oral and intratumour</td>
<td>Mouse</td>
<td>Abe et al. (1991)</td>
</tr>
<tr>
<td>OCT</td>
<td>Enhancement of vasoconstrictor response</td>
<td>200 ng/kg BW</td>
<td>Osmotic minipump</td>
<td>Rat</td>
<td>Shimisawa et al. (1993)</td>
</tr>
<tr>
<td>KH1060</td>
<td>Immunosuppressive in autoimmune disease</td>
<td>3000 ng/kg BW</td>
<td>i.p. Injection</td>
<td>Rat</td>
<td>Lillevang et al. (1992)</td>
</tr>
<tr>
<td>EB1089</td>
<td>Inhibition of breast cancer growth</td>
<td>0.5–2.5 μg/kg BW</td>
<td>Oral</td>
<td>Rat</td>
<td>Colston et al. (1991; 1992b)</td>
</tr>
<tr>
<td>EB1089</td>
<td>Inhibition of PTH-related peptide production and prevention of related hypercalcaemia</td>
<td>100 850 ng/kg/24 h</td>
<td>Permanent i.p. infusion</td>
<td>Rat</td>
<td>Haq et al. (1993)</td>
</tr>
<tr>
<td>MC903</td>
<td>Treatment of psoriasis</td>
<td>25 100 μg vehicle</td>
<td>Topical application</td>
<td>Human</td>
<td>Kragballe et al. (1991)</td>
</tr>
<tr>
<td>MC903</td>
<td>Treatment of cutaneous metastatic breast cancer</td>
<td>100 μg/g vehicle</td>
<td>Topical application</td>
<td>Human</td>
<td>Bower et al. (1991)</td>
</tr>
<tr>
<td>MC903</td>
<td>Inhibition of breast cancer growth</td>
<td>50 μg/kg BW</td>
<td>Oral application</td>
<td>Rat</td>
<td>Colston et al. (1992b)</td>
</tr>
<tr>
<td>MC903</td>
<td>Reduction of HIV-related psoriasis</td>
<td>30 μg/day</td>
<td>Topical application</td>
<td>Human</td>
<td>Gray et al. (1992)</td>
</tr>
<tr>
<td>CB966</td>
<td>Inhibition of breast cancer growth</td>
<td>1 μg kg BW</td>
<td>Oral</td>
<td>Rat</td>
<td>Colston et al. (1991)</td>
</tr>
<tr>
<td>RO 24-2637</td>
<td>Immunosuppressive in transplantation of cardiac allografts</td>
<td>150 200 μg/animal</td>
<td>i.p. Injections</td>
<td>Mouse</td>
<td>Lemire et al. (1992)</td>
</tr>
<tr>
<td>RO 23-7553</td>
<td>Increased survival of mice with myeloid leukaemia</td>
<td>0.8–1.6 μg animal</td>
<td>i.p. Injections</td>
<td>Mouse</td>
<td>Zhou et al. (1990)</td>
</tr>
<tr>
<td>26,27-HF-1.25</td>
<td>Treatment of uroemic patients on haemodialysis</td>
<td>0.05–0.3 μg/day</td>
<td>Oral</td>
<td>Human</td>
<td>Nishizawa et al. (1991)</td>
</tr>
<tr>
<td>26,27-HF-1.25</td>
<td>Treatment of hypoparathyroidism</td>
<td>0.35–35 μg/kg BW</td>
<td>Oral</td>
<td>Rat</td>
<td>Nakatsuka et al. (1992)</td>
</tr>
<tr>
<td>24-F-1.25</td>
<td>Reduction of incidence of parturient paresis</td>
<td>250 μg</td>
<td>Pellet implantation</td>
<td>Cow</td>
<td>Goff &amp; Horst (1989)</td>
</tr>
</tbody>
</table>

OCT (22-oxacalcitriol) Chugai Pharmaceuticals Co., Ltd., Japan; MC903 (Calcipotriol), KHI1060, EB1089, and CB966 LeO Pharmaceuticals Products Ltd A/S, Denmark; RO 24-2637, 26-(26,27-hydroxy-16-ene-vitamin D₃), RO 23-7553, 26-(26,27-dihydroxy-16-ene-23,yne-vitamin D₃), and 24-F-1.25 (24-fluoro-1.25(OH)₂D₃) Hoffmann-La Roche, Inc., USA; 26,27-HF-1.25 (26,27-hexafluoro-1.25(OH)₂D₃) Sumitomo Pharmaceutical Co. Ltd., Japan.

* No studies are mentioned which merely examined effects on serum calcium and urinary calcium.
† Duration of administration, diet, and effects on serum calcium varies between the various studies.

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23-yne-D₃ clearly indicate systemic effects. This compound increased survival time of mice inoculated with fulminant, moderate or slowly progressive leukaemia significantly, while no hypercalcaemia was observed (Zhou et al., 1990). In contrast, the control group treated with a 16-fold lower dose of 1,25-(OH)₂D₃ developed mild hypercalcaemia. The reason for enhanced antitumour activity of 1,25-(OH)₂16-ene-23-yne-D₃ over calcaemic action is unknown, although one can speculate that the 23-yne conformation decreases its susceptibility for C24-oxidation of the side-chain.

An enhanced in-vivo antitumour activity relative to 1,25-(OH)₂D₃, without causing marked hypercalcaemia, has also been described for other analogues (Table 1) (Colston et al., 1991). In this respect it is interesting that the serum half-life of the potent antitumour analogue EB 1089 was comparable to 1,25-(OH)₂D₃ (2.8 vs 2.4 hours), while EB 1089 has nevertheless a threefold lower calcaemic activity of the potent antitumour analogue EB 1089 was comparable to 1,25-(OH)₂D₃ (2.8 vs 2.4 hours), while EB 1089 has nevertheless a threefold lower calcaemic activity of vitamin D analogues with properties especially capable of modulating non-classic actions of 1,25-(OH)₂D₃. As discussed, several mechanisms at the cellular level may be candidates to explain the properties of the new analogues. However, more detailed structure and function studies of the vitamin D molecule, together with studies on the molecular mechanisms of gene activation and possible tissue-specific effects of vitamin D, will be needed to assess whether it is indeed possible to develop analogues devoid of calcaemic actions. Finally, it has to be emphasized that in-vitro screening of new analogues provides insufficient data for selecting potential candidates for future clinical applications. It is clear that intestinal absorption, metabolism (both target-specific and general), and transport to target cells (binding proteins) are major determinants of the ultimate effect of an analogue in vitro.

Conclusions

The picture that emerges from the recent research with vitamin D analogues clearly shows the possibility of developing analogues with properties especially capable of modulating non-classic actions of 1,25-(OH)₂D₃. As discussed, several mechanisms at the cellular level may be candidates to explain the properties of the new analogues. However, more detailed structure and function studies of the vitamin D molecule, together with studies on the molecular mechanisms of gene activation and possible tissue-specific effects of vitamin D, will be needed to assess whether it is indeed possible to develop analogues devoid of calcaemic actions. Finally, it has to be emphasized that in-vitro screening of new analogues provides insufficient data for selecting potential candidates for future clinical applications. It is clear that intestinal absorption, metabolism (both target-specific and general), and transport to target cells (binding proteins) are major determinants of the ultimate effect of an analogue in vitro.

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