1,25-Dihydroxyvitamin D₃ Receptors and Resistance: Implications in Rickets, Osteomalacia, and Other Conditions

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Rickets or osteomalacia may arise from a defect anywhere in the bone mineralization pathway, which begins with the process of vitamin D activation, includes input of minerals to the bloodstream, and ends with accumulation of mineral crystals in osteoid. This chapter focuses upon the actions of vitamin D, and particularly the central role of the receptor for 1,25-dihydroxyvitamin D₃ [1,25(OH)₂D₃] in bone mineralization and in other processes. I will give an overview of the normal structure, normal regulation, and normal actions of 1,25(OH)₂D₃ receptors. Then I shall review the implications of defects that disturb responsivity to 1,25(OH)₂D₃ in all tissues as opposed to defects that disturb responsivity to 1,25(OH)₂D₃ in selected tissues.

NORMAL 1,25(OH)₂D₃ RECEPTORS

The Normal Structure of 1,25(OH)₂D₃ Receptors

The amino acid sequence of the 1,25(OH)₂D₃ receptor has been deduced from molecular cloning data (1,2), revealing that the 1,25(OH)₂D₃ receptor belongs to the family of v-ERB-A related proteins. This family of DNA-binding gene-regulators includes the protein encoded by v-ERB-A, one of the two oncogenes associated with avian ERythroBlastosis (3) (thus ERB) (the other is v-ERB-B, which codes for a truncated form of the receptor for epidermal growth factor), the receptors for steroid (glucocorticoid, progesterone, estrogen, androgen, and mineralocorticoid) hormones, the receptors for thyroid hormones, the receptors for retinoic acid, receptors for as yet unidentified ligands, and two Drosophila embryo pattern-related proteins (4,5). Most members of this family have a ligand-binding domain at the carboxy-terminus, a DNA-binding domain [with two cysteine “zinc fingers,” subdomains each with four cysteine residues that may complex zinc] in the middle, and a less evolutionarily conserved amino-terminus of highly variable size. Two acidic regions
in the amino- and carboxy-terminal regions (of glucocorticoid receptors) show a transcriptional activating function (6).

Normal Regulation of $1,25(OH)_2D_3$ Receptors

$1,25(OH)_2D_3$ receptors can be regulated by $1,25(OH)_2D_3$ itself in either a positive (homologous up regulation) or negative (homologous down regulation) direction (7).

Alternately, they can be regulated by other molecules, of which glucocorticoids are good examples. Glucocorticoids increase $1,25(OH)_2D_3$ receptor capacity (heterologous up regulation) in tissues of the rat, whereas glucocorticoids decrease this capacity (heterologous down regulation) in the mouse (8).

Unidentified mediators have important effects on $1,25(OH)_2D_3$ receptors in important situations. For example, cellular concentrations of the $1,25(OH)_2D_3$ receptor are regulated by unknown factors during the cell cycle (9) and (in a tissue-specific manner) during ontogeny (10).

Normal Activation of $1,25(OH)_2D_3$ Receptors

Steroid receptors are thought to be functionally inactive until binding to a steroid hormonal agonist initiates the process of receptor "activation." Activation may be a multistep process, including subunit dissociation, dimerization, and repositioning onto chromatin. The ligand-binding site of the $1,25(OH)_2D_3$ receptor has tightest affinity for $1,25(OH)_2D$, among the natural calciferol metabolites. However, since other metabolites, such as $25(OH)D$, circulate at far higher concentration than $1,25(OH)_2D$, the ligand-binding site could normally be activated by a mixture of metabolites.

Normal Locations and Actions of $1,25(OH)_2D_3$ Receptors

$1,25(OH)_2D_3$ receptors are found in many normal tissues, including duodenal mucosa, parathyroid chief cell, osteoblast, osteocyte, dental papilla, renal proximal tubule, renal distal tubule, pancreatic beta cell, pituitary thyrotrope, mammary epithelium, epidermis, outer root sheath cell of hair follicle, T lymphocyte, monocyte, placenta, and nervous system (11–13).

$1,25(OH)_2D_3$ receptors are believed to act principally by regulating (up or down) concentrations of selected mRNAs. And most of the important actions of $1,25(OH)_2D_3$ are mediated by receptors in duodenal mucosa that induce increased flux of calcium from mucosa to serosa. In fact, the antirachitic actions of calciferols may result only from their actions on intestinal calcium transport. This has been supported by experiments in which vitamin D-deficient animals have achieved major improvement, if not complete normalization, of bone mineralization by regimens
that have compensated only for the deficiency in calcium input from intestine (14-16).

Potentially important roles of 1,25(OH)\(_2\)D\(_3\) receptors in other tissues include differentiation and activation of osteoclast precursors (17), differentiation of immature monocytes and myelocytes (18,19), actions on the osteoblast, differentiation of epidermal keratinocytes (20), and suppression of mitosis and hormone synthesis by the parathyroid cell (21). 1,25(OH)\(_2\)D\(_3\) receptors may mediate all actions of the calciferols. However, there have been suggestions that 24,25(OH)\(_2\)D\(_3\) exerts unique actions, not shared with 1,25(OH)\(_2\)D\(_3\), in fetal cartilage/chondrocyte (21a). If a separate receptor with greater specificity for 24,25(OH)\(_2\)D\(_3\) is confirmed, the concept of absolute requirement for only a 1,25(OH)\(_2\)D\(_3\) receptor would need modification. However such a subclass of calciferol receptor need not have any role in the pathophysiology of rickets or osteomalacia.

1,25(OH)\(_2\)D\(_3\) RECEPTORS IN DIVERSE RACHITIC STATES

1,25(OH)\(_2\)D\(_3\) receptors have not been analyzed in detail in any rachitic states, excepting those thought to result from defects in the receptor itself (see below). But since this receptor has such a critical role in intestinal absorption of calcium, it is essential to recognize its highly diverse functions in distinct rachitic processes.

States with Deficiency of Vitamin D, 25(OH)D, or 1,25(OH)\(_2\)D

In all states with low serum 1,25(OH)\(_2\)D, rickets and osteomalacia reflect principally deficient activation of receptors for 1,25(OH)\(_2\)D\(_3\) in duodenal mucosa.

States with Tissue Non-Selective Resistance to 1,25(OH)\(_2\)D\(_3\)

These states, believed to result from specific defects in the 1,25(OH)\(_2\)D\(_3\) receptor, also reflect deficient activation of 1,25(OH)\(_2\)D\(_3\) receptors and are discussed in detail below.

States with Deficiency of Calcium or Phosphorus But Not of Vitamin D Metabolites

In states with isolated deficiency of calcium (22) or isolated deficiency of phosphorus (23), there is a reactive increase of renal 25(OH)D\(_3\) 1α-hydroxylase activity. This leads to high serum 1,25(OH)\(_2\)D, increased activation of 1,25(OH)\(_2\)D\(_3\) receptors, and an increased fractional absorption of calcium by the intestine. This homeostatic activation of 1,25(OH)\(_2\)D\(_3\) receptors explains an apparent paradox—the association
of absorptive hypercalciuria with a rare form of osteomalacia (from hereditary renal wasting of phosphate) (23,24).

States with Combinations of Calciferol Metabolite Deficiency, 1,25(OH)$_2$D$_3$ Receptor Defects, and Mineral Deficiency

Special roles for acquired defects in 1,25(OH)$_2$D$_3$ receptor function have been suggested in the rickets or osteomalacia associated with intestinal immaturity (suggested lack of 1,25(OH)$_2$D$_3$ receptor in intestine during early development (10,25)) and X-linked hypophosphatemia [perhaps an acquired defect in 1,25(OH)$_2$D$_3$ receptor function (26,27)].

1,25(OH)$_2$D$_3$ RESISTANCE: CATEGORIES AND NOMENCLATURE

Hormone resistance is a state with subnormal hormone effect despite normal or high hormone concentrations. For 1,25(OH)$_2$D$_3$ the normal effect is stimulation of duodenal calcium transport, and a deficient effect is usually manifested as hypocalcemia, secondary hyperparathyroidism, and impaired skeletal mineralization (rickets and/or osteomalacia). Since 1,25(OH)$_2$D$_3$ also has certain effects on tissues outside the duodenal mucosa, a definition of 1,25(OH)$_2$D$_3$ resistance can be applied to these tissues as well.

Herein, the term *tissue non-selective* resistance to 1,25(OH)$_2$D$_3$ encompasses defects thought to disturb the 1,25(OH)$_2$D$_3$ response pathway uniformly in all tissues (28). The terms *generalized* or *widespread* resistance are often used to convey a similar meaning. Such defects are believed to arise, for the most part, from mutations causing the receptor gene to code for a deficient or abnormal receptor in all 1,25(OH)$_2$D$_3$ target tissues. This is sometimes termed *pseudo-deficiency rickets type II* or *hereditary vitamin D-dependent rickets type II*. The latter term is no longer appropriate, because some patients with this disorder are unable to respond to (i.e., they are not "dependent" upon) even the highest possible doses of calciferols (see below).

The term *tissue selective* resistance to 1,25(OH)$_2$D$_3$ refers to defects that disturb responsivity in some but not all target tissues (28). The terms *local* or *focal* resistance have a similar meaning but tend to imply anatomic regionalization that is not applicable to dispersed targets such as myeloid tissue. Tissue selective defects may or may not directly disturb the structure or numbers of 1,25(OH)$_2$D$_3$ receptors. For example, a defect in cell differentiation may render a potential 1,25(OH)$_2$D$_3$ target tissue unresponsive to many factors including 1,25(OH)$_2$D$_3$.

While tissue non-selective (generalized) resistance to 1,25(OH)$_2$D$_3$ is usually hereditary, tissue selective (localized) resistance to 1,25(OH)$_2$D$_3$ is usually not hereditary.
Clinical Features of Tissue Non-Selective Extreme Resistance to 1,25(OH)$_2$D$_3$

The syndrome of tissue non-selective extreme resistance to 1,25(OH)$_2$D$_3$ shows autosomal recessive transmission of hypocalcemic rickets with very high serum 1,25(OH)$_2$D (particularly during treatment) and variable degrees of alopecia (29,30) (Fig. 1). Only 30 or so families with this disorder have been reported, an indication of extreme rarity. Most cases have been recognized in regions near the Mediterranean, presumably reflecting the frequency of consanguineous mating in the populations showing this disorder. The typical presentation is a baby with hypocalcemia, rickets, and alopecia. Alopecia (reported in about half of cases) may date to birth.

**FIG. 1.** A 5-year-old boy with hereditary resistance to 1,25(OH)$_2$D. He was unable to sit without support at age 5 (A). Though unresponsive to high doses of calciferols, he showed improvement two years later following treatment with high doses of calcium orally (B). Though weakness and deformity improved, alopecia worsened during treatment. From Marx SJ (30).
or may evolve between ages 2 to 12 months. Some children have also shown a papular skin eruption.

Defects in the 1,25(OH)\(_2\)D\(_3\) response pathway have been demonstrated in many tissues from these subjects including mitogen-activated mononuclear cells, cultured dermal fibroblasts, cultured epidermal keratinocytes, and cultured bone cells. Though this disorder allows evaluation of the consequences of defective 1,25(OH)\(_2\)D\(_3\) receptors in all potential target tissues (31), alopecia has been the only documented abnormality beyond that expected in the duodenal mucosa. The alopecia has been found only in cases with the most extreme resistance to 1,25(OH)\(_2\)D\(_3\), as judged from the degree of abnormality in the relation between 1,25(OH)\(_2\)D\(_3\) and calcium in serum during successful or unsuccessful therapy (32) (Fig. 2). This syndrome seems likely to result from mutations in selected domains of the gene for the 1,25(OH)\(_2\)D\(_3\) receptor; all cases have shown receptor defects \textit{in vitro} (see below). The type of defect (of receptor interaction with 1,25(OH)\(_2\)D\(_3\) or with other intracellular molecules) has had no clear relation to clinical expression. Apparently each class of defect can occur as a spectrum of severity, and the severity of the defect seems to be the determinant of clinical expression (32,33).

**Cellular Basis for Tissue Non-Selective Extreme Resistance to 1,25(OH)\(_2\)D\(_3\)**

Most cases with undetectable hormone-binding nevertheless show normal amounts of receptor protein by immunoassay (34), suggesting selective defects in the hormone-binding region of the receptor without 1,25(OH)\(_2\)D\(_3\) receptor protein deficiency. In a small minority of cases there has been a suggestion of receptors with normal hormone-binding capacity but abnormally low hormone-binding affinity (35).
Several cell lines with normal hormone-binding to receptors have shown abnormal receptor-binding to DNA cellulose (36,37), suggesting defects in the receptor's DNA-binding domain. And several kindreds have shown normal receptor-binding to hormone and to DNA but apparently deficient translocation of receptor from cytosol to nucleus (38). Some kindreds with extreme resistance to 1,25(OH)$_2$D$_3$ may have 1,25(OH)$_2$D$_3$ receptor gene defects that impair expression and apparently not structure of the receptor protein. This could account for the defects in several kindreds with cells showing deficient hormone-binding capacity but normal hormone-binding affinity (38). It seems likely that cells from each kindred will show a defect in the 1,25(OH)$_2$D$_3$ receptor gene. This hypothesis has been supported by important studies in two kindreds with defects in receptor binding to DNA: cells from each kindred showed a different mutation in the first or second "zinc finger" region. Either finger being believed to be critical in receptor interaction with DNA (39).

Cells with presumed defects in the gene for the 1,25(OH)$_2$D$_3$ receptor have proved useful to establish that the 1,25(OH)$_2$D$_3$ receptor can stimulate cyclic guanosine monophosphate rises within 1-3 minutes after hormone addition (40) and to prove that the normal receptor couples in strikingly different ways to mitogenic versus anti-mitogenic actions of 1,25(OH)$_2$D$_3$ (41).

Mutations affecting proteins encoded by 1,25(OH)$_2$D$_3$-responsive genes could, in theory, cause tissue non-selective or tissue selective defects in 1,25(OH)$_2$D$_3$ action. No protein critical for all 1,25(OH)$_2$D$_3$ responses [other than the 1,25(OH)$_2$D$_3$ receptor itself] has been established though several candidate proteins exist. For example, mutations in a vitamin D-dependent calcium-binding protein [coded for by at least two distinct genes in the rat (42)] might cause tissue selective or tissue non-selective resistance to 1,25(OH)$_2$D$_3$. And a mutation causing deficiency of carbonic anhydrase II is one cause of osteoclast-selective resistance to 1,25(OH)$_2$D$_3$ (43) (i.e., osteopetrosis, see below).

**1,25(OH)$_2$D$_3$ RESISTANCE: TISSUE SELECTIVE DEFECTS**

There are several types of disorders (see 1-4 below) wherein one or few tissues, potentially responsive to 1,25(OH)$_2$D$_3$, show a defect that this hormone might overcome.

1. In osteopetrosis (generally an hereditary disorder in humans), a spectrum of osteoclast dysfunctions causes high serum 1,25(OH)$_2$D (44) and resistance to certain skeletal actions of 1,25(OH)$_2$D$_3$ (45); it is unclear if other actions of 1,25(OH)$_2$D$_3$ are also compromised, but 1,25(OH)$_2$D$_3$ actions on monocytes or on precursors common to osteoclasts and leukocytes could be deficient. This resistance probably does not reflect defects in structure or numbers of 1,25(OH)$_2$D$_3$ receptors but rather a series of dysfunctions that prevent the osteoclast from being activated by many factors including 1,25(OH)$_2$D$_3$. One specific etiology (associated with osteopetrosis, renal tubular acidosis, and basal ganglia calcification) has been identified as absent car-
bonic anhydrase II (43), an enzyme not believed to be regulated by 1,25(OH)$_2$D$_3$ (46).

2. Chronic renal failure is associated with secondary hyperparathyroidism; this reflects, in part, impaired production of 1,25(OH)$_2$D$_3$ and resultant hypocalcemia. However, parathyroid hyperplasia can occur in uremia even when hypocalcemia is prevented (47). This could reflect, at least in part, an increase in the "set-point" for suppression of the uremic parathyroid cell by calcium (48). A second defect of 1,25(OH)$_2$D$_3$ metabolism in chronic renal failure is decreased capacity of 1,25(OH)$_2$D$_3$ receptor binding in parathyroid cells (49); this could interfere with normal 1,25(OH)$_2$D$_3$ suppression in these cells and thereby account for parathyroid hyperplasia even with normal ionized calcium in blood.

3. A role for 1,25(OH)$_2$D$_3$ in skin disorders has been sought because of the identification of 1,25(OH)$_2$D$_3$ receptors (13) and responses (20) in epidermal tissues and because of the association of alopecia with tissue non-selective resistance to 1,25(OH)$_2$D$_3$ (34). Psoriasis is but one example of a disorder associated with lack of differentiation or increased proliferation in the basal layers of the epidermis; and one study suggested that cultured psoriatic fibroblasts from involved or even from uninvolved skin sites show partial resistance to the antiproliferative action of 1,25(OH)$_2$D$_3$ (50).

4. Efforts to show unique roles for 1,25(OH)$_2$D$_3$ receptors in cancer tissues have been unrewarding. However, many cancers express 1,25(OH)$_2$D$_3$ receptors, and these can mediate antiproliferative effects of 1,25(OH)$_2$D$_3$ in vitro (51).

The concept of tissue-selective defects need not imply complete tissue selectivity. For example, a systemic dysfunction such as uremia might well impair 1,25(OH)$_2$D$_3$ responsivity in many tissues and at many metabolic steps. But the defect in one tissue (for example, the skin) may be the most strikingly expressed. Most importantly, the relatively normal 1,25(OH)$_2$D responsivity of the duodenum dictates that secondary hyperparathyroidism and a bone mineralization defect are not caused by tissue selective (i.e., local) 1,25(OH)$_2$D$_3$ resistance.

**THERAPY: TISSUE NON-SELECTIVE RESISTANCE TO 1,25(OH)$_2$D$_3**

Tissue non-selective (i.e., generalized) resistance to 1,25(OH)$_2$D$_3$ is usually responsive to high doses of calciferols with or without calcium supplementation (29,30). Therapy can often normalize calcium homeostasis including rickets, but alopecia never improves and may even worsen (Fig. 1).

There are three grades of disease severity that correspond to three strategies for therapy (Table 1).

**Severity Grade 1.** Cases with moderate severity respond to calciferols [D$_2$, D$_3$, or 25(OH)D$_3$] alone as substrates for endogenous production of large amounts of 1,25(OH)$_2$D. Such patients can sustain extraordinarily high serum 1,25(OH)$_2$D in part because of deficient 1,25(OH)$_2$D receptor-mediated feedback inhibition of renal 25(OH)D 1α-hydroxylase.
TABLE 1. Treatment strategy for generalized resistance to 1,25(OH)₂D₃, subdivided by grade of severity of presentation

<table>
<thead>
<tr>
<th>Presenting severity</th>
<th>Calcium intake</th>
<th>Calciferol analogue</th>
<th>Daily dose</th>
<th>Therapy mechanism</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 1—moderate</td>
<td>Normal diet (1 gm) without supplement</td>
<td>D₂ or D₃, 25(OH)D₃</td>
<td>0.5–5 mg, 30–300 μg</td>
<td>Calciferol analogue generates high 25(OH)D, substrate for 1α-hydroxylation</td>
</tr>
<tr>
<td>Grade 2—intermediate</td>
<td>Normal diet plus supplement (2 gm)</td>
<td>1α,25(OH)₂D₃, 1α(OH)D₃, Dihydrotachysterol</td>
<td>5–60 μg, 5–60 μg, 4–40 mg</td>
<td>High circulating equivalent of 1α,25(OH)₂D gives higher and invariant calcium absorption</td>
</tr>
<tr>
<td>Grade 3—worst</td>
<td>Ultra-high oral supplement (3–7 gm) or i.v. (0.3–1 gm)</td>
<td>None</td>
<td>—</td>
<td>Fixed high calcium input to blood</td>
</tr>
</tbody>
</table>

These doses of calcium and calciferols encompass the ranges needed for initial treatment ("hungry bones" phase) or long-term maintenance. A patient may improve rather suddenly in severity category around the end of the "hungry bones" phase. These doses are typical for adults. Calcium and calciferol doses are similar in children and adults.

As total daily calcium intake (diet plus supplement) in grams of elemental calcium (elemental calcium is only 10–40% of the calcium salt, depending upon the calcium preparation).

Severity Grade 2. In cases with intermediate severity, endogenous production of 1,25(OH)₂D cannot be sufficiently increased, and therapy requires extremely high doses of 1α-hydroxyl analogues of the calciferols (Fig. 2) (and when serum 1,25(OH)₂D activity is thereby fixed at one level, high dose calcium supplementation is an important adjunct to avoid fluctuations from variable dietary calcium content).

Severity Grade 3. In the most severely affected cases with a complete absence of intestinal response to very high doses of 1,25(OH)₂D₃ (Fig. 3), major benefit can still derive from therapy with high doses of calcium orally (52) or intravenously (Fig. 4) (53–55).

The criteria for establishing that the disorder is severe enough for inclusion in severity grade 3 have not been given careful attention. The usual criterion is lack of response to a therapeutic trial, but certain conditions must be met: (i) the trial must attain circulating concentrations of calciferol metabolites equivalent to 2000 pg/ml 1,25(OH)₂D; (ii) the high drug levels must continue for at least a month; and (iii) monitored response indices must be appropriate (because bone remineralization may take several months, during which time changes in intestinal flux of calcium may not be accompanied by detectable changes in serum calcium). Preferred (i.e.,
requiring much shorter drug exposure) criteria of response can be derived by evaluating the actions of calciferols at the intestinal mucosa (e.g., fractional calcium absorption); however, such evaluations are complex, requiring either metabolic balance studies or the use of isotopes [radioactive or stable (55)].

These three treatment categories and their mechanisms of effect serve to emphasize that even in the occurrence of tissue non-selective resistance to 1,25(OH)₂D₃, treatment directed selectively at the duodenal target tissue is beneficial or even curative for the bone mineralization defect. Even patients with the most severe defects can achieve normal or near-normal bone mineralization if heroic measures are taken to normalize calcium and phosphorus levels in blood. These latter findings support animal investigations suggesting that 1,25(OH)₂D₃ receptors in bone do not have an important role in the normal bone mineralization process (14–16).

As is true for therapy of nutritional vitamin D deficiency and deficient 25(OH)D₃ 1α-hydroxylase (pseudo-vitamin D deficiency type I), the early stages of treatment
in generalized resistance to 1,25(OH)$_2$D$_3$ involve catch-up mineralization ("hungry bones"). Large fluxes of calcium into bone may continue for 1–3 months without concomitant changes in serum calcium or parathyroid hormone. The doses of calciferol analogue and/or calcium that can be effective during this phase may prove to be excessive during maintenance therapy, when calcium flux into bone is determined principally by the normal process of growth and senescence. And a patient can improve into a less severe category of "severity grade" (see above) on completion of catch-up mineralization.

**THERAPY: TISSUE SELECTIVE RESISTANCE TO 1,25(OH)$_2$D$_3$**

Tissue selective (localized) resistance to 1,25(OH)$_2$D$_3$, unlike generalized resistance, cannot be expected to be ameliorated by systemic calcium. The recognition of abnormalities with features of tissue selective dysfunction in 1,25(OH)$_2$D$_3$ re-
sponse provides insight into what have seemed to be novel approaches (see 1–4 below) to calciferol pharmacotherapy of non-osteomalacic disorders.

1. Incomplete activation of osteoclasts by high doses of 1,25(OH)₂D₃ has been accomplished in several unusual patients with osteopetrosis (56); low calcium diet seems essential to avoid excessive intestinal absorption of calcium at these high 1,25(OH)₂D₃ doses.

2. Intermittent high dose intravenous administration of 1,25(OH)₂D₃ has been used in uremic hyperparathyroidism (57). By this protocol a relatively selective suppression of the parathyroid may be attained without a calcemic effect.

3. Calciferols have shown promise in therapy of the hyperproliferative skin lesions of psoriasis; calciferols have been given either topically (58,59) or orally (59) in protocols designed to avoid high calcium concentrations in urine or blood. The latter goals during oral therapy were approached through use of moderately high oral doses of 1,25(OH)₂D₃ at night, a time when the duodenal lumen contains little calcium (59) (see chapter by C. Arnaud).

4. Extensive efforts are under way to explore the highly potent "differentiating" actions of calciferols on immature myeloid cells (18,19). 1,25(OH)₂D₃ and 1α(OH)D₃ each prolonged survival (in some protocols without inducing hypercalcemia) in an animal model of myelocytic leukemia (60). Drug development programs have focused thus far upon modification of the C-17 side chain (61–63) (Table 2). One goal of such programs is to develop calciferol analogues with anti-neoplastic effects (on myeloid cells) without significant calcemic effect.

The unifying feature of all these seemingly novel approaches to calciferol pharmacotherapy for tissue selective resistance to 1,25(OH)₂D₃ is the effort to target a 1,25(OH)₂D₃ analogue to a selected tissue (Table 3). This effort activates 1,25(OH)₂D₃ receptors locally without perturbing systemic calcium homeostasis and serves to

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**TABLE 2. 1,25-Dihydroxyvitamin D₃[1,25(OH)₂D₃] analogues showing selective potency for differentiation of monocyte or myelocyte precursors**

<table>
<thead>
<tr>
<th>Reference</th>
<th>1,25(OH)₂D₃ analogue</th>
<th>Differentiation assay potency&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Osteoclast assay potency&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Dn/Oc&lt;sup&gt;d&lt;/sup&gt; Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>61</td>
<td>24-homo-1,25(OH)₂D₃</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>62</td>
<td>22-oxa-1,25(OH)₂D₃</td>
<td>10</td>
<td>0.5</td>
<td>20</td>
</tr>
<tr>
<td>63</td>
<td>Δ-22,26 27-cyclopropyl-1,24(OH)₂D₃</td>
<td>1</td>
<td>0.05</td>
<td>20</td>
</tr>
</tbody>
</table>

<sup>a</sup> From Marx SJ (28).

<sup>b</sup> Measured in different laboratories, using different indices and different cell lines. Data are, therefore, only roughly comparable.

<sup>c</sup> Measured in different laboratories with highly differing assays that vary from calvarial organ culture to calcemic response in vivo. Data are, therefore, only roughly comparable.

<sup>d</sup> Dn/Oc, differentiation assay potency/osteoclast assay potency.
TABLE 3. Summary of pharmacotherapy for disorders with tissue selective resistance to 1,25(OH)2D3

<table>
<thead>
<tr>
<th>Disease</th>
<th>Tissue with 1,25(OH)2D3 resistance</th>
<th>Treatment protocol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteopetrosis</td>
<td>Osteoclast</td>
<td>1,25(OH)2D3 orally, low calcium</td>
</tr>
<tr>
<td>Uremia</td>
<td>Parathyroid</td>
<td>1,25(OH)2D3 intravenous pulses</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Epidermis</td>
<td>1,25(OH)2D3 topically</td>
</tr>
<tr>
<td>Psoriasis</td>
<td>Epidermis</td>
<td>1,25(OH)2D3 orally at night</td>
</tr>
<tr>
<td>Leukemia</td>
<td>Myeloid</td>
<td>1,25(OH)2D3 analogs (experimental)</td>
</tr>
</tbody>
</table>

* Modified from Marx SJ (28).

compensate for a defect that may not even implicate the 1,25(OH)2D3 receptor at all.

SUMMARY AND CONCLUSIONS

The 1,25(OH)2D3 receptor mediates all or most of the actions of calciferols. As such this receptor has a controlling role in intestinal calcium absorption and must be understood for its varying contributions to the pathophysiology of all states of defective mineralization.

Extreme resistance to 1,25(OH)2D3 in all tissues (i.e., generalized or tissue non-selective resistance) is usually an hereditary disorder reflecting mutations, presumed in the 1,25(OH)2D3 receptor gene. The severity of the underlying defect determines the treatment strategy: (i) vitamin D or 25(OH)D3 alone for the mildest cases, (ii) a 1α(OH) analogue of vitamin D together with supplemental calcium for cases of intermediate severity, or (iii) no calciferol but ultra-high dose calcium alone for the most severe defects. Each of these treatment strategies overcomes the most important defect, which is deficient 1,25(OH)2D-dependent flux of calcium from intestine to blood.

Resistance to 1,25(OH)2D3 can also be defined to explain tissue selective abnormalities. These latter abnormalities may or may not directly implicate the 1,25(OH)2D3 receptor gene. Treatment protocols have been developed to deliver 1,25(OH)2D3 analogues to the dysfunctional tissues without a simultaneous systemic calcemic effect. Therapy with a 1,25(OH)2D3 analogue activates 1,25(OH)2D3 receptors locally and, thereby, can compensate for the defect.

While calcium (usually given orally) is a critical co-factor for calciferol therapy in tissue non-selective resistance to 1,25(OH)2D3, calcium (to the point that it can cause hypercalciuria or hypercalcemia) is generally the limiting factor for (i.e., deleterious to) calciferol actions in tissue selective resistance to 1,25(OH)2D3.

ACKNOWLEDGMENT

Portions of this chapter have been previously published (28).
REFERENCES


125(OH)2D3 RECEPTORS AND RESISTANCE


DISCUSSION

*Dr. Holick:* Do your conclusions imply that calcitriol could have a place in the cure of baldness? In fact, it doesn’t work. To place this into perspective, it is interesting that in a vitamin D-deficient rat, brought up through two generations of D-deficient mothers there is no defect in hair follicle development. The same holds true in D deficiency in humans, where there is no evidence of any hair loss or alopecia. So do calcitriol receptors apparently present in the lining cells of the hair follicle, have a physiologic function?

*Dr. Marx:* It is possible that failure to observe alopecia in vitamin D deficiency is because the “deficiency” at the level of receptor activation is incomplete, in contrast with the genetic aspects in receptor function where there is probably one one-thousandth or less of the normal degree of receptor activation. Hereditary severe resistance to 1,25(OH)2D3, as a model, represents a situation to evaluate the role of these receptors in many tissues, such as the osteoclast precursor. I believe that, although 1,25(OH)2D3 may stimulate differentiation of many cell
types effectively in vitro, other factors can accomplish this normally in vivo (even in the complete absence of 1,25(OH)\(_2\)D\(_3\) effect). In contrast, there seems to be an obligatory role for an effect of 1,25(OH)\(_2\)D\(_3\), albeit at a very low "basal" level, in vivo in the hair follicle.

**Dr. Delvin:** Concerning the ubiquity of these vitamin D receptors, do VDD 2 patients have any problems in insulin handling or in immunological responses?

**Dr. Marx:** This question was addressed in considerable detail in a study by Hochberg et al. (see chapter reference 31). They did dynamic tests of insulin, TSH, prolactin, growth hormone, and testosterone secretion in this patient group and found no abnormalities excepting those attributable to hypocalcemia itself. We ourselves have done detailed immunologic testing by looking for skin reactivity to standard allergens in one such patient. Cutaneous reactivity is a good test of much of the immune system, and our patient showed normal responses (unpublished data).

**Dr. Pettifor:** There are some reported cases of what appear to be acquired vitamin D resistance. How do you explain this on a molecular basis?

**Dr. Marx:** It is essential to distinguish calcitriol resistance, which is only one cause of vitamin D resistance. I am only aware of one case for which the former was documented rather well (1). This was a 50-year-old man with a 5-year history of progressively symptomatic osteomalacia. The patient had hypocalcemia and secondary hyperparathyroidism. Maintenance therapy was ultimately established with 8\(\mu\)g/day 1\(\alpha\)(OH)D\(_3\), which is 5–10 times the usual dose for therapy of hypoparathyroidism. Unfortunately serum 1,25(OH)\(_2\)D was not reported, and skin fibroblasts were not studied. If one could really document "acquired" tissue nonselective resistance to 1,25(OH)\(_2\)D, the most likely mechanisms would be through circulating factors, such as antibodies, that inactivate a critical element of the 1,25(OH)\(_2\)D response pathway; the most vulnerable elements in the bone mineralization pathway could be the receptor for 1,25(OH)\(_2\)D\(_3\), surface components on duodenal mucosal cells, or surface components on osteoblasts. Clearly, this is highly speculative.

**Dr. Pettifor:** On a practical level, in cases of suspected calcitriol resistance, what would be the screening test that you would recommend? Fibroblast culture with 24-hydroxylase activity or what?

**Dr. Marx:** The patients are so rare that we have not tried to focus on development of a rapid and specific diagnostic test. Koren et al. (2) have advocated use of 1,25(OH)\(_2\)D\(_3\) inhibition of [\(^3\)H]-thymidine incorporation in mononuclear cells from fresh blood. This assay required 24–48 hours, in contrast to studies on cultured skin fibroblasts which require 6 weeks or much more, plus complex and expensive laboratory support for tissue culture. I believe that the most practical general screening test is a therapeutic trial. If a patient requires high calciferol doses after 3 or more months and still has high 1,25(OH)\(_2\)D levels in blood, the diagnosis is nearly unequivocal.

**Dr. Pettifor:** There is a variability of responses to 1,25(OH)\(_2\)D in patients with end-organ resistance. When one gets down to the lower levels, where they require perhaps five or ten micrograms of 1,25(OH)\(_2\)D\(_3\), are we dealing with the same syndrome, basically?

**Dr. Marx:** Blood level of 1,25(OH)\(_2\)D is more relevant than maintenance dose orally. Such modestly increased maintenance doses have really not been documented. However, I do think there is a continuum of disease. The milder cases are not being recognized because they are "self-treated" through maintenance of high basal serum 1,25(OH)\(_2\)D levels by endogenous production, apparently without accompanying secondary hyperparathyroidism or osteomalacia. We have documented this phenomenon in two obligate heterozygotes (both parents of the same child with hereditary severe resistance to 1,25(OH)\(_2\)D\(_3\)) (unpublished data).

**Dr. David:** I have little personal experience with patients with 1,25-dihydroxyvitamin D
resistance having only one patient in care. This child is actually 7 years old and has a very severe form with complete alopecia. From this limited experience I would like to make two comments. First, I can confirm that large daily oral calcium doses are really very effective in case of severe resistance. Our patient has been treated with 3 or 4 grams of calcium per day for the past 3 years and has been very much improved. The second comment is that I have been surprised by the fact that the plasma PTH levels were only moderately elevated in our patient, while he had severe chronic hypocalcemia. I looked at the literature and found that in many of the published observations there are little information on the PTH levels and usually they appear to be also only moderately elevated. Therefore I wonder if it could be possible that the very high circulating 1,25-dihydroxyvitamin D levels which are present in these patients, may exert a negative feedback on the PTH secretion, which would suggest that there may exist some tissue selective differences in the resistance to 1,25(OH)2D.

Dr. Marx: I agree completely with your observations. One problem is that no one center has studied many patients, and it is difficult to pool results from diverse PTH RIAs.

Dr. Arnaud: I have also observed that patients with hypocalcemic rickets may have apparently very mild elevations of PTH values. By appropriate fractionation it is clear, however, that there is a much higher ratio of biologically active over biologically inactive fragments than in patients with primary hyperparathyroidism or in normal individuals. To use a normal range generated with a middle molecule assay is, thus, inappropriate in rachitic patients.

REFERENCES