

REVIEW

The development of a bone- and parathyroid-specific analog of vitamin D: 2-methylene-19-Nor-(20S)-1 α ,25-dihydroxyvitamin D₃

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The goal of synthetic chemists in the vitamin D field has been to produce an analog(s) of 1 α ,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) that is selective for a specific function. The accumulation of structure/function information has led to the synthesis of two analogs that are both selective and more potent than 1,25-(OH)₂D₃, that is, 2-methylene-19-nor-(20S)-1 α ,25-dihydroxyvitamin D₃ (2MD) and 2 α -methyl-19-nor-(20S)-1 α ,25-dihydroxyvitamin D₃ (2AMD). *In vivo*, the efficacy of 2MD is approximately equal to that of 1,25-(OH)₂D₃ in intestinal calcium transport but is 30- to 100-fold more active in bone mobilization. *In vitro*, 2MD supports new bone synthesis at 10⁻¹² M, whereas 1,25-(OH)₂D₃ is active at 10⁻⁸ M. Similarly, 2MD is two orders of magnitude more potent than 1,25-(OH)₂D₃ in stimulating osteoclastogenesis and osteoclastic bone resorption. 2MD also markedly increases bone mass and bone strength of ovariectomized female rats. In postmenopausal women, 2MD significantly increases markers of both bone formation and resorption but has minimal effect on bone mass. Thus, in patients who are undergoing primarily remodeling rather than modeling (rat), the increased resorption largely counteracts the increased bone formation. So far, 2MD has not been tested for reduction of fractures in this population. However, its selectivity includes the parathyroid gland. Thus in the 5/6-nephrectomy model of chronic renal failure, 2MD is much more potent than currently available vitamin D compounds used to suppress secondary hyperparathyroidism of renal failure without causing hypercalcemia. It is currently in phase 2B trials in patients on dialysis.

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Introduction

Following the isolation, identification and demonstration of the importance of 1 α ,25-dihydroxyvitamin D₃ (1,25-(OH)₂D₃) as the hormonal form of vitamin D, syntheses not only of the 1,25-(OH)₂D₃ but also of variations in that structure were carried out in an attempt to reduce the undesirable hypercalcemic effect of the native hormone.^{1,2} Many of these analogs have not been thoroughly tested to determine whether they have selective activity. Further, the assays in laboratories differ, and thus a comparison of their biological activities is difficult. Therefore, no attempt will be made in this review to compare the activity of 2-methylene-(20S)-19-nor-1 α ,25-dihydroxyvitamin D₃ (2MD) with the activities of analogs other than 1,25-(OH)₂D₃ itself.

A major emphasis was placed on producing an analog that could suppress cancer because of the finding that 1,25-(OH)₂D₃ could suppress growth of cancer cells *in vitro* and promote

differentiation.^{1,3-5} Many attempts were made to prepare analogs that would not increase serum calcium but instead would possess the ability to suppress growth of cancer cells *in vitro*.⁶⁻⁸ Although this was certainly successful, it is not clear whether *in vitro* suppression of cancer growth could be demonstrated *in vivo*. So far, no vitamin D analog has emerged with that property without producing lethal hypercalcemia. In fact, suppression of cancer growth by 1,25-(OH)₂D₃ or an analog *in vivo* has not been uniformly demonstrated.⁹ As discussed in the recent Dietary Reference Intakes Report, the relationship between vitamin D and cancer is far from clear.⁹

However, there is the possibility that modifications in the 1,25-(OH)₂D₃ structure may bring about selective activity on certain biochemical systems. One successful development has been the Leo Pharmaceutical compound, MC-903, also known as Dovonex.^{10,11} When applied topically, this compound clearly

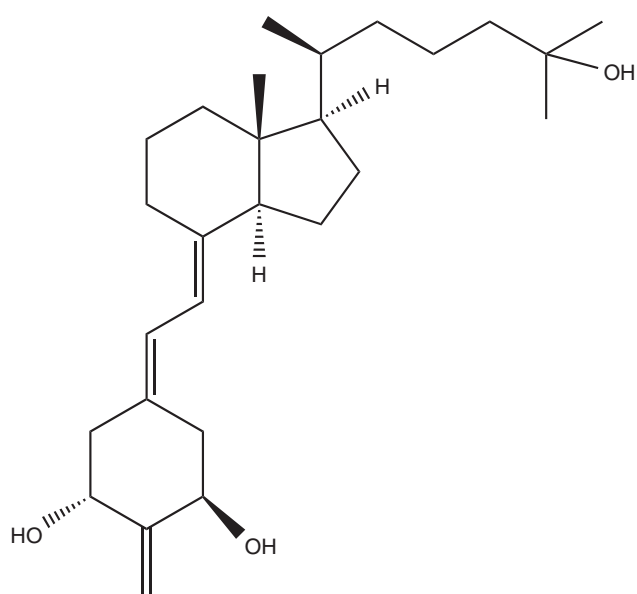
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suppresses proliferation of the keratinocyte and is used as a therapy for the disease psoriasis. This compound does not produce hypercalcemia except under extreme circumstances because it is rapidly metabolized and eliminated with a lifetime of less than a few minutes in the blood stream.^{11,12} Thus, Dovonex is selective only when provided topically, and is ineffective when provided systemically.^{11,12} On the other hand, Chugai Pharmaceuticals' compound, 1,25-dihydroxy-22-oxavitamin D₃ (OCT), when given systemically does suppress secondary hyperparathyroidism, but because it is rapidly metabolized and eliminated it has less of a calcemic effect.¹³ In both cases, the selectivity stems either from the route of administration or from rapid metabolism.

In order for an analog to function selectively, it must be able to alter the vitamin D receptor (VDR) in a differential way. The VDR is a single receptor found in many organs of the body.^{14–16} Thus, it might be expected that a targeted specific form of 1,25-(OH)₂D might influence the structure of the VDR in such a way that it would bind certain coactivators and not others and may lend specificity with regard to target gene responses. Rochel *et al.*^{17–18} and subsequently Vanhooke *et al.*¹⁹ have crystallized the ligand-binding domain (LBD) of the VDR with different analogs. Both groups found that, even with analogs of very divergent structures and activities, the crystalline structure of the LBD is unchanged by the ligand. On the basis of the X-ray structure, it is not possible to see how an analog can be selective in its actions. However, recently the LBD has been studied in solution using nuclear magnetic resonance spectroscopy, in which, in fact, analogs do have different effects on the solution structure of the receptor.²⁰ It is, therefore, feasible that an analog might alter the VDR structure such that it selectively binds coactivators or corepressors that might result in selective activity. This idea has not yet reached fruition, but there is evidence *in vivo* that there could be analogs with such

selective activity. One such analog is 2MD (**Figure 1**).²¹ So far, we have not been able to translate these findings into a mechanism that would reveal why 2MD has preferential activity on bone or the parathyroid gland.²⁰ 2MD and 1,25-(OH)₂D₃ bind the VDR with equal affinity.²¹ However, when given *in vivo* by intravenous injection, 2MD is 30–100 times more effective than 1,25-(OH)₂D₃ in mobilizing calcium from bone.²² In the same animals, its activity is either equal to or even slightly less than that of 1,25-(OH)₂D₃ in inducing intestinal calcium transport by the everted sac method of measuring this system (**Figure 2**).²² As bone calcium mobilization is believed to be the result of the vitamin D hormone interacting with the receptor in the osteoblast to bring about the transcription of the RANK ligand and as the RANK ligand then stimulates the osteoclasts to mobilize bone, this initial result seemed to suggest that 2MD might be selective for activity on the osteoblast. When tested in human osteoblasts in culture, 2MD proved to be two orders of magnitude more effective than 1,25-(OH)₂D₃ in osteoclastogenesis and in inducing osteoclast-mediated bone resorption.²² Furthermore, when tested in the synthesis of new bone by the osteoblast, it also proved to be two or three orders of magnitude more effective than the native hormone.^{21,22} This surprising result raises the question of whether vitamin D compounds are in fact bone anabolic. Until very recently, it had been believed that the primary, if not only, role of the vitamin D hormone in bone was to elevate calcium and phosphorus to levels that cause mineralization of the skeleton, but there was no strong evidence that increased bone synthesis occurred in response to the vitamin D hormone. However, the work of Xue *et al.*²³ provided secure evidence that in fact 1,25-(OH)₂D₃ does have bone anabolic activity in CYP27B1- and parathyroid-ablated mice. This encouraging result led to the testing of whether 2MD given to female, ovariectomized rats might in fact show an increase in bone synthesis, and hence an increase in



2-Methylene-19-nor-(20S)-1 α ,25-Dihydroxyvitamin D₃ (2MD)

Figure 1 Structure of 2MD.

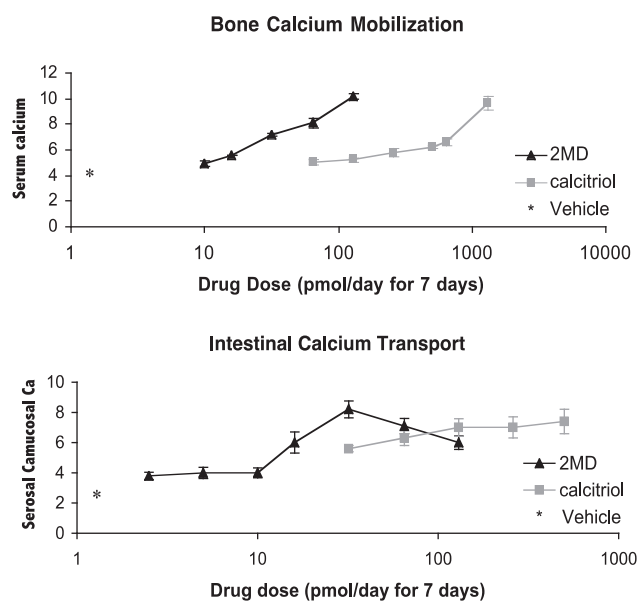


Figure 2 *In vivo* selective activity of 2MD in vitamin D-deficient rats. Rats (male) were placed on a vitamin D-free purified diet containing 0.47% calcium/0.3% phosphorus for 4 weeks, and then placed on the same diet devoid of calcium (0.02%) for 3 weeks and dosed for 7 days. Four hours after the last dose, animals were killed to determine intestinal calcium transport by the everted sac method, and serum calcium was used to determine bone mobilization (see Shevde *et al.*²² for details).

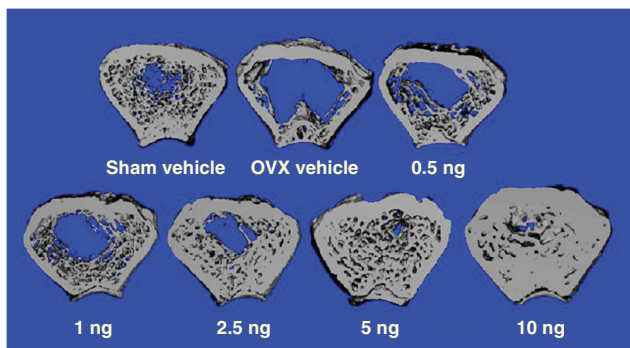


Figure 3 Cross-sections of femoral metaphysics from ovariectomized female rats on the indicated daily dose of 2MD. Sections of femoral metaphysics of 1-mm thickness were scanned by a pQCT machine.

bone mass. Retired female breeders were ovariectomized and treated with 2MD, and the results were quite marked, showing an increase in bone mass.^{24,25} That 2MD is bone anabolic in the rat is evident by the experiments by Pfizer's scientists (**Figure 3**).²⁵ However, it is well known that the rat is primarily a bone-modeling organism and has very little bone remodeling, whereas the human skeleton is largely a remodeling skeleton.²⁶ Thus, it was not certain whether the bone mass effect in the rat would be translated into an increase in bone mass in humans. When a 1-year clinical trial in postmenopausal women with osteopenia was carried out, 2MD markedly increased the bone biomarkers of both bone resorption and formation.²⁷ However, little change in bone mass occurred. But again in rats, bone strength, determined by three-point bending measurements and other measurements, showed that bone strength was improved by 2MD.²⁴ In developing toxicity studies in monkeys and rats in preparation for clinical trials, a clinical research organization found that the bones of animals given 2MD were much harder than normal and were difficult to cut. This suggested that 2MD increased bone turnover and as a result may have provided increased bone strength. However, the fact that 2MD did not increase bone mass in postmenopausal women with osteopenia prevented the expenditure of \$1 billion for a human fracture study required by the FDA for approval. Thus, it is unknown whether 2MD might be beneficial in postmenopausal osteoporosis. Because of the position taken by the FDA that a fracture study in postmenopausal women will be required for approval, it is not clear whether this question will ever be answered.

One of the findings that resulted from the preclinical studies done in the Pfizer laboratory is that 2MD preferentially localizes in the parathyroid glands, as shown in **Figure 4**. Furthermore, when tested in the 5,6-nephrectomy model of rats, 2MD seemed extremely effective without raising serum calcium in suppressing secondary hyperparathyroidism (**Figure 5**). In fact, in the 5,6-nephrectomy model, 2MD appears 10 times more active than 1 α -hydroxyvitamin D₂ (Hectorol) or 19-nor-1 α ,25-dihydroxyvitamin D₂ (Zemplar) in the suppression of parathyroid hormone levels (Zella J *et al.*, unpublished results). Thus, 2MD has been introduced into the dialysis world as a possible replacement of vitamin D analogs already in use. A clinical trial underway will largely determine whether this compound provides a significant improvement over existing therapies

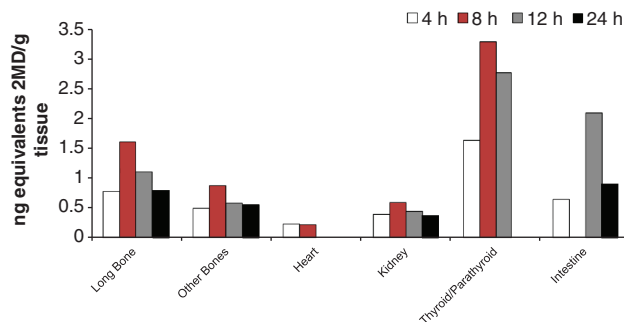


Figure 4 Localization of 2MD in the parathyroid glands, bone and intestine. Radiolabeled 2MD (26,27-³H; 160 Ci/mmol) was given, sections of tissue were taken and radioactivity was detected by autoradiography.

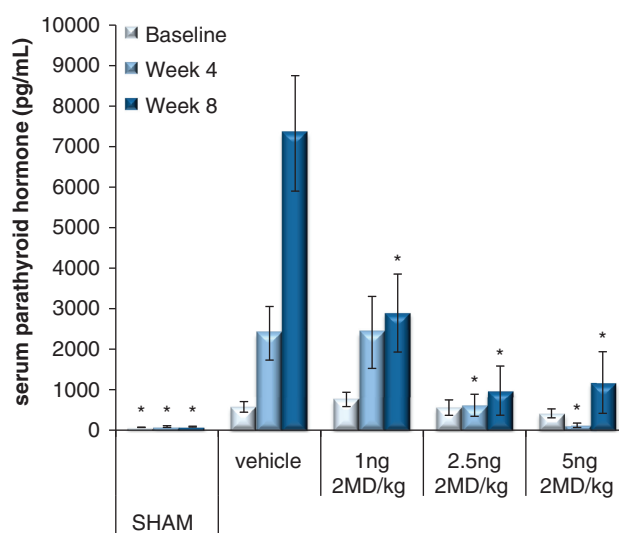


Figure 5 Suppression of secondary hyperparathyroidism in 5/6-nephrectomized rats. The nephrectomies were carried out as described in the study by Brown *et al.*¹³ Following 4 weeks of recovery, the indicated daily oral dose or vehicle was administered. Parathyroid hormone (PTH) measurements were taken at the indicated times.

and whether it will be useful in controlling secondary hyperparathyroidism.

Summary

2MD is a promising analog of 1,25-(OH)₂D₃ that may prove to be an improvement in the treatment of secondary hyperparathyroidism in dialysis patients and may actually be an excellent agent for improving bone strength in postmenopausal women, thereby aiding in the treatment of osteoporosis.

Conflict of Interest

The author is the CEO and President of Deltanoid Pharmaceuticals, Inc., which is developing 2MD.

Acknowledgements

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