D-Hormone Derivatives for the Treatment of Osteoporosis: From Alfacalcidol to Eldecalcitol

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Abstract: Many readers may have only a vague idea about vitamin D. This is made complicated, in part, because it is also expressed with suffixes such as vitamin D_2 or vitamin D_3. Otherwise the prefix of "active" is also occasionally used. Vitamin D is often referred to as an important nutrient for calcium intake, especially for growing children and the elderly. On the other hand, it serves as a therapeutic drug for osteoporosis and psoriasis. Recent studies have suggested an association with a number of diseases such as cancer, diabetes and Alzheimer's disease. The author has been involved in the research and development of vitamin D applications for over 25 years, and has often witnessed even world-class experts confuse the two roles – vitamin and hormone – of "substance" D. Assuming some readers are not familiar with vitamin D, D hormone or osteoporosis, an outline of vitamin D and D hormone is delineated with a particular focus on the treatment of osteoporosis. Furthermore, development of alfacalcidol as the first prodrug of D hormone (calcitriol) and eldecalcitol as a characteristic new D hormone derivative and basic relationship between calcemic activity and effect on bone in vitamin D (cholecalciferol), D hormone (calcitriol/alfacalcidol), and a new D hormone derivative (eldecalcitol) are introduced.

INTRODUCTION

Many readers may have only a vague idea about vitamin D. This is made complicated, in part, because it is also expressed with suffixes such as vitamin D_2 or vitamin D_3. Otherwise the prefix of "active" is also occasionally used. Vitamin D is often referred to as an important nutrient for calcium intake, especially for growing children and the elderly. On the other hand, it serves as a therapeutic drug for osteoporosis and psoriasis. Recent studies have suggested an association with a number of diseases such as cancer, diabetes and Alzheimer's disease. The author has been involved in the research and development of active vitamin D applications for over 25 years, and has often witnessed even world-class experts confuse the two roles – vitamin and hormone – of "substance" D. It is also perhaps not well known that Japanese researchers from both industry and academia have consistently been global leaders in this field, from the initial academic studies of basic research to the development of new pharmaceuticals. This article discusses the application of D hormone derivatives for therapies and the work based on the achievements of earlier researchers. Assuming some readers are not familiar with vitamin D, D hormone or osteoporosis, an outline of vitamin D and D hormone is delineated with a particular focus on the treatment of osteoporosis.

FROM VITAMIN D TO D HORMONE [1,2]

Rickets, a bone disease, has been common since ancient times, particularly in Europe. A therapeutic factor that is known to be contaminated in oleum morrhuae, was initially thought to be vitamin A, however, subsequent studies found that this substance was different from vitamin A and was named vitamin D. The code "D" was derived from the fact that it was found next to vitamin C. Currently, six forms of vitamin D are known, from D_2 to D_7, identified by the difference in side chains. Although there are reasonable grounds for missing D_1, these are not discussed in this paper. The common formation process of all types of vitamin D involves previtamin D. Cleavage of the B-ring in provitamin D takes place by exposure of the 5,7-diene sterol (provitamin D) to ultraviolet light, followed by subsequent thermal isomerization of previtamin D to produce vitamin D, the secosteroid. As the A-ring of vitamin D inverts from the original steroid framework during thermal isomerization, α and β, which show the configuration of substituents of the A-ring of vitamin D are now opposite to the normal convention (according to the IUPAC nomenclature) (Fig. (**1**)).

Of the six forms of vitamin D, D_2 through D_7, only D_2 and D_3 have been found to be biologically active based on the elucidation of activation pathways. Accordingly, vitamin D usually refers to vitamin D_2 and/or vitamin D_3. Vitamin D_3, cholecalciferol, is derived from 7-dehydrocholesterol on the skin following ultraviolet irradiation by sunlight, or taken in by eating seafood, among other foods. Vitamin D_2, ergocalciferol, is also taken orally by consuming fungi such as Japanese mushrooms. In several countries including the US, it is added to milk. While partially synthesized in the skin, oral ingestion is essential for vitamin D_2 and vitamin D_3 intake, which are referred to as native vitamin D. Within this context, substance "D" is definitely positioned as a vitamin. Vitamin D intake or synthesized in the skin metabolizes to 25-hydroxyvitamin D (25OHD) following 25-hydroxylation of the side chain in the liver. Since this hydroxylation in the liver is not strictly controlled contrary to hydroxylation at the 1α-position in the kidney, 25OHD serves as a vitamin D reserve in the body, stably circulating in the blood for a long

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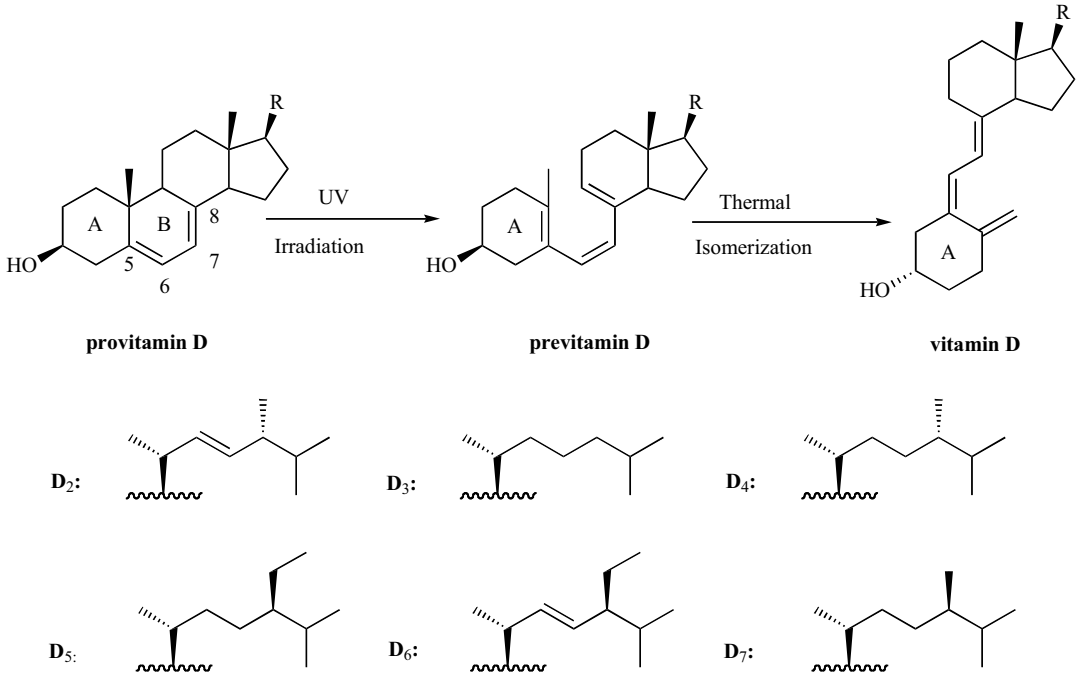

Fig. (1). Biosynthesis of vitamin D from provitamin D *via* previtamin D and side chain structure of vitamin D_2-D_7.

time while binding to the specific vitamin D binding protein (DBP) [3]. Hence the 25OHD concentration in the blood represents vitamin D sufficiency.

25OHD is absorbed, as needed, into proximal renal tubules from the lumen by megalin, hydroxylated at the 1α-position by 1α-hydroxylase, and ultimately metabolized to 1α,25-dihydroxyvitamin D [1,25$(OH)_2$D] [4]. The metabolites are delivered as active vitamin D to targeted organs such as the small intestine, bone, kidney and parathyroid gland which enables various biological responses after binding to vitamin D receptors (VDR) [5]. Initially, active vitamin D was recognized as a substance involved in the calcium metabolism. The main actions, which include absorption of calcium from the small intestine, mobilization of bone mineral, and reabsorption of calcium by the kidney, increase the concentration of calcium in the blood and, are needed to successfully maintain the body's calcium level. Studies have revealed that VDR exists in tissues and organs all over the body, such as skin, brain, and muscles, and is not limited to the small intestine, kidney, bone, and parathyroid gland. This suggests that vitamin D deficiency might be associated with various diseases as outlined in the introduction. Hydroxylation in the kidney is strictly controlled by parathyroid hormone (PTH) or fibroblast growth factor-23 (FGF-23). Hence, 1,25$(OH)_2$D is referred to as active vitamin D. In this context, substance "D" is completely positioned as a hormone, which is why 1,25$(OH)_2$D is referred to as D hormone. The generic name of active vitamin D_3, 1,25$(OH)_2D_3$, is calcitriol, while that 25OHD_3 is calcifediol. Fig. (**2**) illustrates the activation pathway of vitamin D_3 (Fig. (**2**)).

DEVELOPMENT OF PRODRUG: ALFACALCIDOL

Patients with renal damage who require artificial kidney dialysis are expected to develop vitamin D deficiency due to a disorder of vitamin D activation caused by insufficient hydroxylation in the kidney. An effective treatment for such individuals with renal impairment is through chemical treatment, specifically the administration of a vitamin D derivative with a hydroxy group at 1α-position. As part of these efforts, 1α-hydroxyvitamin D_3 (generic name: alfacalcidol) was developed in Japan in 1981 as the first prodrug of calcitriol for medical use. As described above, hydroxylation in the liver is comparatively loose, and alfacalcidol is activated to calcitriol in the body. In addition, it has been revealed that this activation takes place in the bone (Fig. (**2**)). A key question is why the prodrug alfacalcidol was developed as a pharmaceutical product, rather than the exact substance calcitriol. There are two reasons for this; one is that it was difficult to develop calcitriol for medical use due to issues associated with patent rights; the other – discussed in detail in subsequent paragraphs – is that alfacalcidol had some advantages with respect to the cost of industrial synthesis, compared to calcitriol. Specifically, inexpensive and easily available cholesterol could be used as the starting material because alfacalcidol does not have a hydroxy moiety at the 25-position. Although the initial indications included vitamin D deficiency and hypocalcemia, osteoporosis was added to the list of indications in 1983. Clinically alfacalcidol has been the first-line treatment for osteoporosis in Japan for more than 25 years [6,7].

Osteoporosis is a disease caused by bone resorption overtaking bone formation and the creation of an imbalance between the two processes that is normally due to aging. Women in particular develop the disease more frequently than men because their bone mineral density is rapidly reduced after menopause. For the prevention of osteoporosis, it is very important to ingest enough calcium and get exposure to sunlight in order to form sufficient vitamin D from an

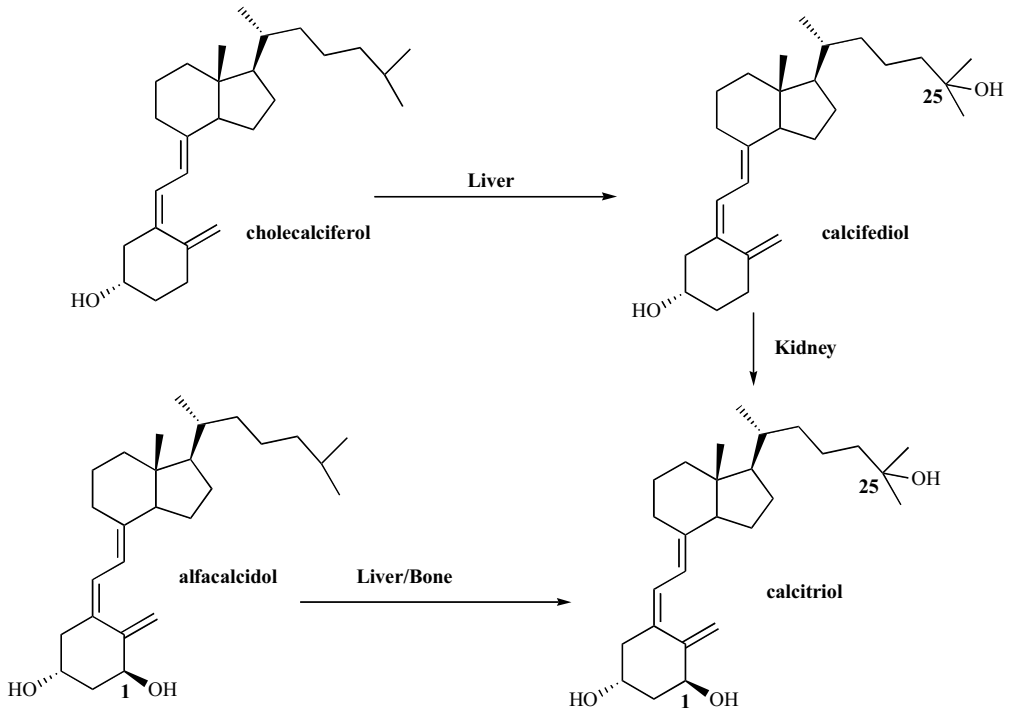

Fig. (2). Activation of vitamin D (cholecalciferol) and prodrug (alfacalcidol) to D hormone (calcitriol).

early age. It is definitely a myth, however, that the administration of calcium and vitamin D is effective for the treatment of fractures in patients with osteoporosis. The administration of high-dose calcium would only be a burden on these advanced-age patients because the VDR level in their small intestine is low. Furthermore, if high-dose vitamin D was administered to them, sufficient D hormone would not be supplied because 25OHD would not be activated due to reduced renal hydroxylase activities. The author and colleagues recently found that the administration of high-dose vitamin D only increases bone strength to a certain level in ovariectomized rats (OVX rats), while D hormone, alfacalcidol, treatment improves both strength and density dose dependently [8]. An explanation for such a phenomenon has not yet been elucidated. It is suggested, however, that only calcitriol is produced from alfacalcidol for blood circulation, while a number of minor metabolites are produced in addition to D hormone from vitamin D. Almost no roles for these metabolites have been clarified. It should be acknowledged that the physiological need of ingesting vitamin D is significantly different from the administration of D hormone for the treatment of osteoporosis.

In Europe and the US, bisphosphonates (e. g., sodium alendronate, sodium risedronate, sodium ibandronate) or selective estrogen receptor modulators (SERM), such as raloxifene, have been mainly used for the treatment of osteoporosis. This is contrary to Japanese clinical practice, which focuses on D hormone therapy [9]. Various reasons for this have been pointed out which includes differences in the history of osteoporosis therapy development, the amount of calcium intake, the ratio of responders to non-responders due to gene polymorphism of VDR, and culture. Of these reasons, the most significant is that in Western countries, the average amount of calcium intake is higher than in Japan, and vitamin D is added to milk and milk products. Hence, in Western countries, vitamin D as a treatment might induce hypercalcemia rather than have therapeutic effects on bone [10]. Currently SERM and bisphosphonates are also gaining ground as an accepted form of therapy in Japan.

Fig. (**3**) shows an example of the industrial synthesis of alfacalcidol. In brief, its features are:

1) inexpensive and easily available cholesterol is used as the starting material

2) 1α-hydroxy group is stereoselectively introduced by opening the α-epoxide

3) introducing a biomimetic method, i. e., ultraviolet irradiation and thermal isomerization used to obtain the same transformation in the skin caused by sunlight following the synthesis of the 5,7-diene segment

If 25-hydroxycholesterol is used, basically a similar reaction scheme would follow but with much higher costs (Fig. (**3**)) [11].

DEVELOPMENT OF NEW D HORMONE DERIVATIVE: ELDECALCITOL

In 1981, basic research found the existence of VDR in various organs and tissues of the body and that D hormone facilitates cell differentiation and antiproliferation activities in tumor cells, while D hormone had been considered to be involved in only calcium metabolism [12]. During that same year, alfacalcidol came onto the market. Following alfacalcidol treatment in patients with vitamin D deficiency or hypocalcemia, there were an increasing number of those with good clinical prognosis of concurrent rheumatoid arthritis or intractable dermatologic psoriasis vulgaris. It was also found that if the dose of alfacalcidol was increased to raise the

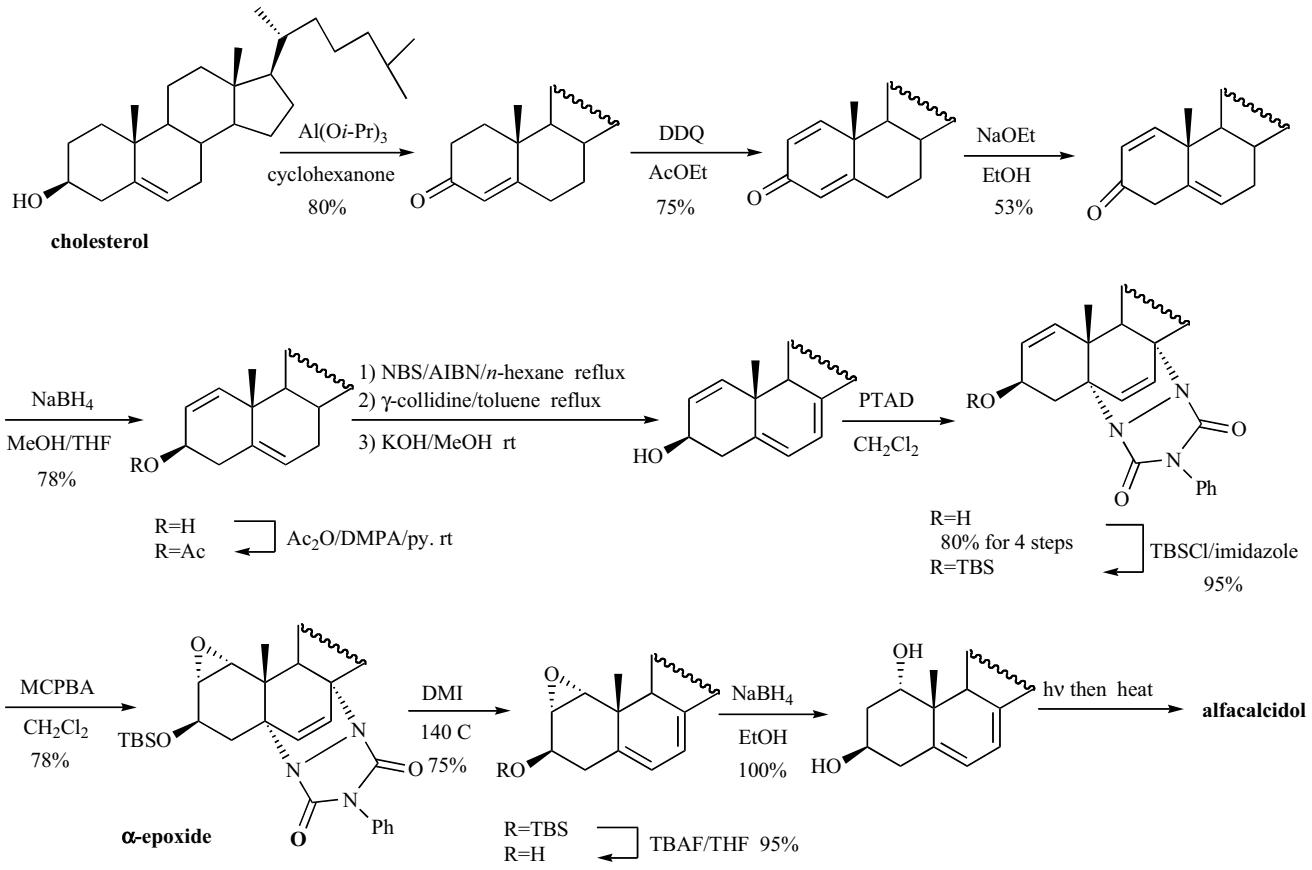

Fig. (3). An example of improved synthesis of alfacalcidol from cholesterol.

therapeutic effect on comorbidities, hypercalcemia developed as an adverse effect before achieving the intended effect. Although specific reasons for the clinical effectiveness of D hormone for psoriasis vulgaris and rheumatoid arthritis were unknown, an increasing number of scientists associated the effect with the cell differentiation and antiproliferation activities found in basic research. Subsequently, many exploratory studies on synthesizing D hormone derivatives with indications of cell differentiation activity *in vitro* and calcemic activity *in vivo* were performed all over the world [13,14]. The obvious focus was placed on discovering derivatives with potent cell differentiation but weak calcemic action. To this aim, a new D hormone derivative was developed in different dosage forms, in which modifications of the side chain was attempted. This resulted in the development of maxacalcitol ointment for the treatment of psoriasis vulgaris and maxacalcitol injection for the treatment of secondary hyperparathyroidism, in author-involved research. These treatments are now used in clinical practice. Please refer to the relevant study for details (Fig. (**4**)) [15].

On the other hand, a D hormone derivative with the completely opposite action to maxacalcitol has been discovered in the modification of the A-ring. In other words, modification of the A-ring led to the discovery of a derivative with weak *in vitro* cell differentiation properties but potent calcemic action in blood *in vivo*. Assuming that potent calcemic activity is accompanied by the intense effect on bone, such effects of a derivative were examined using OVX rats, which found higher effects on bone than alfacalcidol. A series of derivatives were synthesized, in which the α-epoxide (Fig. (**3**)) was utilized as a key intermediate. This compound is also a key intermediate of the industrial scale synthesis of alfacalcidol. It was discovered that nucleophiric opening of the epoxide using alkoxides or carbanions occurs in a regioselective and stereoselective manner in which the nucleophile ends up in the 2β-position. Subsequently, such an approach was applied to α-epoxide with a hydroxy group at the 25 position of the side chain. This resulted in a number of derivatives with various sizes and substituents, such as those with alkoxide or alkyl groups. Finally, eldecalcitol (development code ED-71) with hydroxypropoxy substituent at the 2β-position was selected from these studies which displayed the most intense bone effects in OVX rats (Fig. (**4**)).

In clinical use, the intestinal calcium absorption effect of alfacalcidol becomes active in a daily dosage of 0.25 μg to 0.5 μg and becomes saturated with higher doses, while 0.75 μg to 1 μg of alfacalcidol suppresses PTH and inhibits bone resorption in adults who ingest a normal amount of calcium. These findings suggest a therapeutic threshold for alfacalcidol, wherein bone is intensified. On the other hand, bone resorption is increased at a dosage of 1.5 μg or more, which is the threshold to develop adverse effects. It has been pointed out that D hormone has a narrow therapeutic window. The author and colleagues recently demonstrated that D

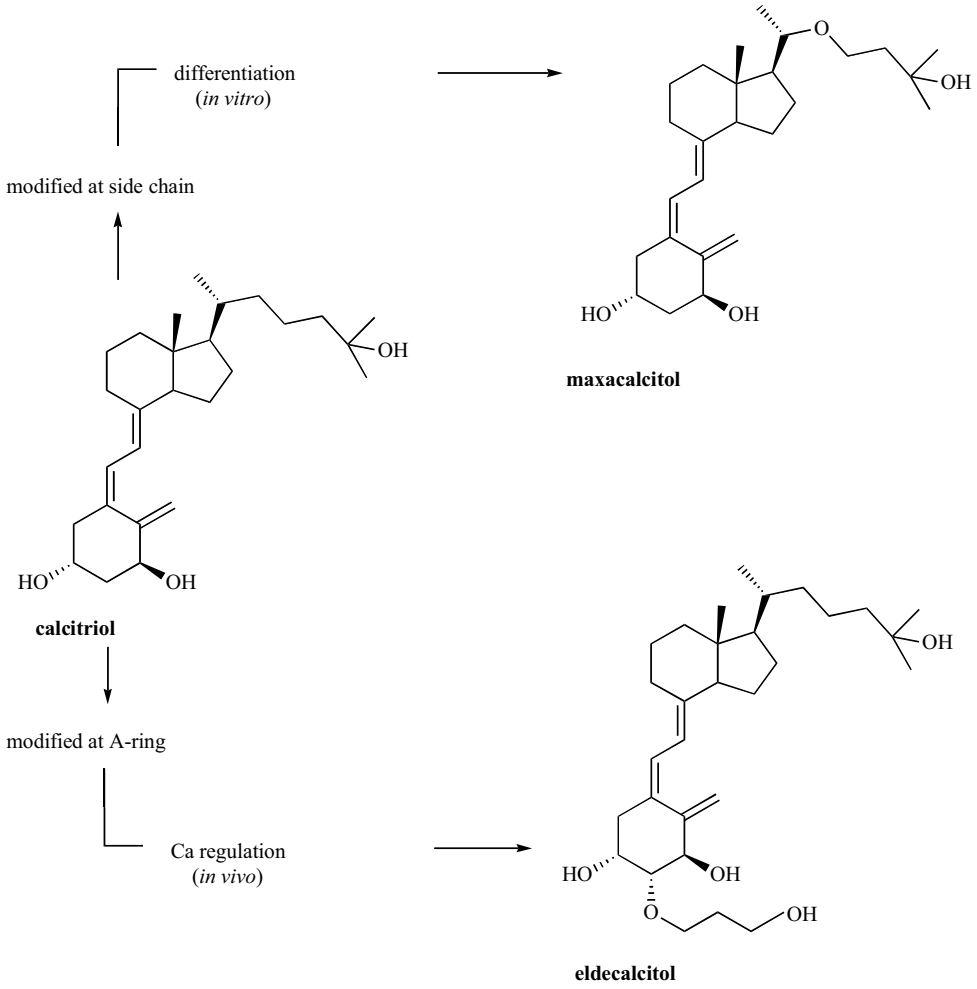

Fig. (4). Characteristic two D hormone derivatives, maxacalcitol and eldecalcitol.

hormone promoted bone formation within the therapeutic threshold in parathyroidectomized rats, not due to intestinal calcium absorption or PTH suppression but due to the direct effect on bone [8]. Eldecalcitol promotes potent bone formation in lower doses than alfacalcidol, has a wider window than alfacalcidol between therapeutic and bone resorption, and is therefore expected to be a useful therapy for osteoporosis [16,17]. Fig. (**5**) illustrates the basic relationship between calcemic activity (serum calcium level) and the effect on bone (increase in bone mineral density, BMD) in vitamin D (cholecalciferol), D hormone (calcitriol/alfacalcidol), and a new D hormone derivative (eldecalcitol) [18]. The potential effect on bone is highest with eldecalcitol followed by calcitriol/alfacalcidol and then cholecalciferol, at doses that induce approximately the same level of calcemic activity (Fig. (**5**)). The question is what is the different mode-of-action between calcitriol/alfacalcidol and eldecalcitol. The demonstrated physiological features of eldecalcitol are that it exists in the blood for a long period due to a strong affinity for DBP compared with calcitriol [19] and that intrinsic PTH suppression in patients with osteoporosis is low in clinical practice compared with calcitriol or alfacalcidol [20]. Specific physiological properties and mechanism of action of eldecalcitol are consistently being investigated.

A phase III clinical trial with eldecalcitol in Japan began in 2004 and was completed at the end of 2008. The trial was a randomized, double-blind, comparative study to compare

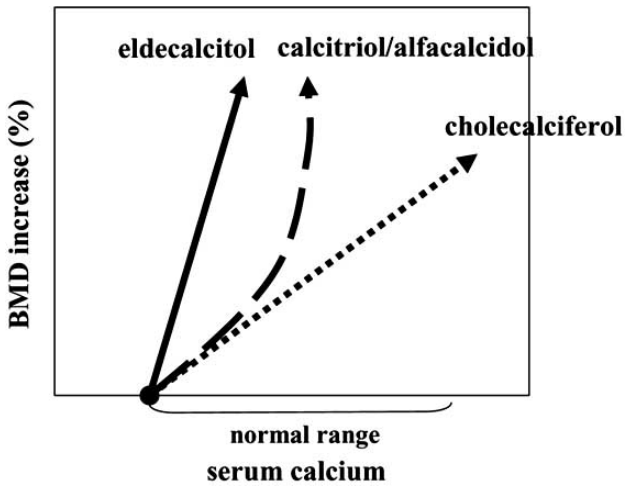

Fig. (5). Basic relationship between calcemic activity (serum calcium level) and effect on bone (increase in bone mineral density, BMD) in vitamin D (cholecalciferol), D hormone (calcitriol/alfacalcidol), and a new D hormone derivative(eldecalcitol).

the efficacy and safety of eldecalcitol with that of alfacalcidol in osteoporotic patients. A total of 1087 patients were randomly allocated to receive a once-daily oral dose of either eldecalcitol (0.75 μg) or alfacalcidol (1 μg), and the incidence of new vertebral fractures in both groups was monitored for a period of 3 years. As a result, patients receiving eldecalcitol showed a significantly lower incidence of bone fractures compared to those receiving alfacalcidol, indicating that eldecalcitol is superior in preventing fractures. The safety profile was similar to that of alfacalcidol, and nothing irregular was observed. The detailed study results will be published soon. Clinical trials for osteoporosis treatment usually require the verification of fracture prevention effect by 3-year administration, which brings enormous costs and requires a long time for development. It is expected, however, that eldecalcitol, as a characteristic new D hormone derivative, will contribute to the treatment of patients with osteoporosis in the near future after the process of application and approval.

FUTURE PROSPECTS

There are still many challenges ahead in attempting to gain a full understanding of the mode-of-action of eldecalcitol with the objective of developing an even more effective and sophisticated pharmaceutical product. This demands the need for new improvements to achieve a more effective and safer D hormone derivative for osteoporosis based on the assessment of its limitations.

Existing D hormone studies based on the recognition of calcitriol as the active metabolite by Japanese scientists, including the author, seem to have paid too much attention to calcitriol since the original research began. In our body, there are many metabolites of vitamin D for which the roles are unknown. They may have important physiological actions such as maintaining homeostasis. Exploration of new medicinal drugs from this point of view can be expressed as follows: "study the past to discover new drugs" which is modeled after an old saying, "study the past to learn new things." In fact, an idea was presented recently that calcitriol is responsible for calcium metabolism whereas the strong binding of calcifediol to DBP is responsible for an anabolic effect on bone [21].

Furthermore, studies have suggested a link between vitamin D and various diseases, such as cancer, diabetes and Alzheimer's disease. The author would like to conclude this article with the expressed hope that a wide range of studies will lead to the future development of various new pharmaceutical products in the industry. "A line-up of various D hormone derivatives as pharmaceutical products" is envisaged. This is the dream and the "romance" of a medicinal chemist who has been deeply involved in this field of research for more than a quarter of a century [22].

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