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Review Article

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Combined therapies in osteoporosis: Bisphosphonates and Vitamin D-hormone analogs

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Abstract

During the last two decades, the development of new, highly effective therapeutics (e.g. bisphosphonates, SERMs, strontium ranelate and PTH) has significantly extended the spectrum of osteoporosis therapy. However, the interest of combining bone-active agents and/or Vitamin D and calcium is still being debated, and is restricted to a very marginal set of compounds (Alendronate and native Vitamin D). On the other hand, Vitamin D-hormone analogs, calcitriol, and alfacalcidol, have repeatedly demonstrated their effectiveness in being valuable alternatives compared to native Vitamin D in this setting. A growing amount of data documents the pre-clinical and clinical efficacies of combinations of bisphosphonates with calcitriol, or with alfacalcidol in primary and secondary osteoporosis. This exhaustive review of the available animal and clinical data aimed at comparing the theoretical with demonstrated absolute and relative benefits of those therapeutic approaches. Most of the pre-clinical and clinical data in PMOP suggest significant, clinical improvements in response to combination therapies versus monotherapies in postmenopausal osteoporosis. As investigated by most of the currently available trials, a daily dose of alendronate 10 mg or a weekly dose of alendronate 70 mg plus alfacalcidol 0.5-1.0 mcg daily seems to surpass other combinations when BMD and bone metabolism markers are considered. A synergy with bisphosphonates in reducing the fracture episodes may lie in the pleiotropic effects of D-hormone analogs on musculoskeletal, immunological and neurological systems. Negative interactions between both drugs have not yet been reported, while a reduction of hypercalcuria episodes has been noted in combination therapies, as compared to monotherapies involving high doses of Vitamin D, calcitriol, or alfacalcidol. Based on the possible reduction of periodic safety checks of calcemia, an improved compliance could then be expected, which would, in turn, generate a better end result. However, to document this, long-term, high quality comparative studies with factorial designs are needed to determine which role this alternative should play in the management of postmenopausal, male, and glucocorticoid-induced osteoporosis.

Keywords: Osteoporosis, Bisphosphonates, Vitamin D, D-hormone Analogs

Background

Greater life expectancy is an achievement of the modern era. However, longevity is inevitably accompanied by an increase in age-related diseases, including osteoporosis-related fractures. The human, economic and social burdens of such fractures are a serious challenge for the actual and future healthcare systems while effective therapeutics at affordable prices are constantly under investigation. During the last two decades, the development of new, highly effective therapeutics (e.g., bisphosphonates, SERMs, strontium ranelate and PTH) has significantly extended the spectrum of osteoporosis management. The reduction of the relative risk for fractures attributable to anti-osteoporosis drugs ranges between 30% and 60%, depending on the type and stage of osteoporosis and the drugs used that has expanded a hypothesis originally restricted to bone mineral density (BMD). In the elderly and in patients treated with glucocorticoids (GCs), frequent falls are accepted as an independent risk factor causing vertebral and non-vertebral fractures[1-3], which is correlated to the observed relative lack of efficacy of some bisphosphonates in reducing hip fractures in very old patients with other risk factors besides low bone mineral densi-

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ty (BMD)[4]. Since Vitamin D deficiency is very common, and given the relatively low cost of such a supplement, combined treatment with a bisphosphonate plus Vitamin D3 has very recently been proposed to and approved by the FDA in postmenopausal osteoporosis (PMOP). However, despite clear demonstrations of their interest in PMOP and glucocorticoid-induced osteoporosis (GIOP) as monotherapies, little is known about the efficacy of a combined treatment using Vitamin D-hormone analogs, alfacalcidol (1-α(OH)D_3), or calcitriol (1,25$(OH)_2$ D_3) and bisphosphonates in this setting.

Alfacalcidol is a synthetically produced, inactive pro-Hormone, metabolized in the liver and other organs into active form, calcitriol[5,6]. Both compounds have been shown to equally decrease bone loss and fracture rates in PMOP, while exhibiting a higher efficacy as compared to native Vitamin D in this setting[7,8]. Alendronate is a second generation bisphosphonate that binds to hydroxyapatite in bones, and acts as an inhibitor of osteoclastic bone resorption. It is used as a standard treatment of osteitis deformans, postmenopausal osteoporosis, and hypercalcemia related to malignancy. An ample amount of evidence supports the use of alendronate as a reference treatment for PMOP and GIOP[9,10].

Theoretical basis for a complementary action of alendronate and Vitamin D-hormone analogs

Fundamental research has shown that the anti-resorbing properties of these two types of drugs are explained by different mechanisms. Alendronate works mainly on matured osteoclasts by reducing their metabolism, activity and number by inducing apoptosis[11-13] and inhibiting enzymes of the mevalonate pathway[14], which in turn inhibits bone resorption. Calcitriol and alfacalcidol decrease osteoclastogenesis by decreasing the pool of osteoclast precursors in the bone marrow, and exhibit a certain degree of anabolic action[15,16]. Although bisphosphonates increase BMD as a result of the inhibition of bone resorption, their ability to increase cancellous bone area measured by histomorphometry in patients with osteoporosis remains debated[17,18]. Vitamin D-hormone analogs have been shown to reduce PTH levels in elderly patients, which is commonly high, as a result of both an increased calcium absorption and an inhibition of the proliferation of the parathyroid gland, as well as by PTH synthesis and release[19], lower the release of pro-inflammatory cytokines, which contribute to osteoclast activation[20-22]. In summary, signalling via the Vitamin D receptor (VDR) tends to stabilize and normalize bone turnover through partial anti-resorptive and anabolic mechanisms while bisphosphonates exert a direct limitation on osteoclastic bone resorption. Based on this concerted action, the response rate of bisphosphonates, notably alendronate, could be optimized by using a combined therapy with Vitamin D-hormone analogs[23]. A growing body of evidence about the effects of alfacalcidol on the number and activity of osteoblasts *in vitro* and *in vivo*, e.g., increased synthesis of bone growth factors (TGF-β; IGF-1 and -2, bone morphogenetic proteins BMPs) and on bone matrix proteins (collagen I, osteocalcin, osteopontin), could, therefore, document a possible balancing of the reduced bone turnover observed when using bisphosphonate therapy[24,25]. Alfacalcidol appears to be active on bone resorption and formation, besides the effects it has on calcium and PTH levels[15,26,27]. The bone anabolic effects of alfacalcidol have been shown *in vivo* in combination with alendronate[28,29]. The clinical outcomes of a combined therapy could, therefore, result in a higher increase in BMD, bone strength, and a further decrease in fracture risk as has been suggested in early animal trials[28-30]. Apart from these bone-related effects, another synergism of the combination might be expected from the effect of Vitamin D-hormone analogs on muscular and neuromuscular systems, and, consequently, the reduction of falls already observed[31-35]. These risk factors are neither positively nor negatively influenced by alendronate[36]. As a result, the combination could translate into a better anti-fracture treatment, as compared to using a monotherapy.

Pre-clinical studies

A pre-clinical study[30] investigated the combined effects of alendronate and alfacalcidol on bone density, bone strength and bone quality in aged ovariectomized (OVX) female rats, and comparing them with monotherapies. The results showed that biochemical markers of bone resorption, like deoxypyridinoline, were significantly decreased when using combined treatments, as compared to respective single treatments, and confirmed the hypothesis of an increased inhibition of bone resorption. Treatment with alendronate alone failed to affect BMD while alfacalcidol increased BMD at the mid-femur only. The combined treatment provided a significantly more marked effect in terms of BMD and bone mechanical strength (mid-femur and L2-L4). Micro-CT and histomorphometric analyses showed that the density of trabecular bone, as measured after using a combined treatment, appeared to be higher than that resulting from a single treatment by showing a better preservation of the bone microarchitecture. These results are in line with those of a recent study suggesting that the bone anabolic effects of alfacalcidol could be enhanced when combined with alendronate[28].

Another study, featuring a similar factorial design, compared the efficacies of risedronate and calcitriol in the prevention of bone loss among OVX rats[29]. No significant difference was noted between the two compounds as to the inhibition of osteoclast maturation, induction of osteoclast recruitment or BMD. However, although OVX-induced bone loss was prevented by variable dosing of either risedronate or calcitriol, OVX rats treated with a combination of both had higher tibial and vertebral BMD values, and significantly increased bone strength in the long bone and vertebra. In combined administration, a significantly higher cancellous bone area was noted, as compared with the groups receiving the monotherapies. Authors postulated that, in combined therapy, calcitriol enhanced the inhibitive effects of risedronate on osteoclast maturation and number while partially counteracting the suppressive effects of the compound on bone formation. The concomitant administration of high doses of risedronate and calcitriol did stabilize serum calcium.

Risedronate was tested with alfacalcidol in a similar model

using OVX rats[37]. The combined administration reduced urinary deoxypyridinoline excretion more than either drug alone. The increase in vertebral strength was higher in the combination therapy than in the monotherapies. Of particular interest was that in the pQCT analysis the additional effect of the combination was produced in cortical and sub-cortical regions more than in the trabecular region, and this effect was mainly responsible for the marked increase in spinal strength.

Etidronate and alfacalcidol markedly increased the serum osteocalcin level, as compared to etidronate alone. In addition, the combination increased bone mass and mechanical strength in OVX rats[38].

In the light of the current available evidence, these findings concerning the complementary effects of different Vitamin D-hormone analogs and bisphosphonates cannot be considered as systematic, i.e., there is no firm evidence that combined treatments with various bisphosphonates and bone-active agents do systematically lead to positive outcomes. Variable effects might be related to different potencies of bisphosphonates in interfering with the mevalonate pathway[39,40], and may be exacerbated when other bone-active agents are present at supraphysiological levels. In these pre-clinical OVX rat models, all five groups of authors concluded that the combined therapy using bisphosphonates with alfacalcidol or calcitriol might be seen as clinically promising in the management of established postmenopausal osteoporosis. However, clinical research has enabled better characterization of the anti-osteoporotic efficacy and safety (notably as concerns the risk of hypercalcemia) of such combination treatments.

Clinical studies: effect of bisphosphonates and Vitamin D-hormone analogs on falls

It has been suggested that BMD and its variations explain only about 30% of the total fracture risk[41]. Falls account for a significant proportion of the remaining explained variances of fracture risk. Indeed, currently the common term "osteoporotic fracture" is focused on bone strength, and does not reflect the fact that 90% of all extravertebral fractures, i.e., hip, humerus, wrist and pelvic fractures, are the result of a fall[1,42-43]. In a recently published study, it was shown that a significant amount of vertebral fractures are fall-related[3]. Therefore, pharmacologic and non-pharmacologic fall prevention should be systematic components of osteoporosis management.

Vitamin D-hormone receptor has been found in skeletal muscles and nerves and may play an indirect role in neuromuscular co-ordination, in addition to the synthesis of muscle proteins[44,45]. Mice from which the VDR gene was deleted suffered a reduction of skeletal muscle fiber size based on an increased expression of myogenic regulation factors (Myf5, Myogenin, E2A) through which the strict regulated differentiation and maturation of muscle cells was disturbed[46]. The fact that a treatment with Vitamin D-hormones of VDR-positive myoblasts *in vitro* down-regulates the aforementioned myoregulating transcription factors, emphasized the role of Vitamin D-hormone and VDR in muscle development[46]. Old age is significantly associated with decreased VDR expression in human skeletal muscle tissue[47]. A positive correlation was found between femoral muscle strength and function and Vitamin D-hormone serum levels in the elderly[48,49]. These results suggest that the age-related and the glucocorticoid/inflammation-induced decline in muscle strength and function and the increase of falls could be in part related to a decrease or alteration of VDR in target organs, and a decrease or resistance to Vitamin D-hormone in serum and/or at receptor levels. These findings were the basis for the rationale for improving muscle function and postural capacity and falls using Vitamin D-hormone analogs[35,50-52].

The rationale for treatment of "sarcopenia" in old age with Vitamin D-hormone analogs has recently been investigated in randomized controlled trials (RCT). In a NIH-sponsored, randomized, placebo-controlled controlled trial, in postmenopausal women aged 65-77 with osteopenia and normal 25(OH)D serum levels, it was shown that a 3-year treatment with calcitriol (0.25 μg twice daily) significantly reduced the rate of falls by 38%[33,53]. A significant reduction of fallers by -55% and falls by -54% in community-dwelling women and men aged 75 years on average with normal serum 25(OH)D levels was determined in another prospective RCT after 9 months' treatment with 1 μg alfacalcidol daily, provided with a total calcium intake of more than 500 mg supplied by the daily diet[32]. In this RCT it was also investigated whether treatment with alfacalcidol could reduce the high risk of falls associated with low creatinine clearance (CrCl). Thirty-six weeks of treatment with alfacalcidol versus placebo (1 μg daily) significantly and safely reduced the risk of falls by up to 71% in community-dwelling elderly men and women with a CrCl of <65 ml/min, which was similar to the fall rates observed in patients with normal CrCl and calcitriol levels[34].

On the basis of available clinical studies, alendronate did not show either a positive or a negative influence on neuromuscular parameters or falls[36]. Therefore, the observed effect of Vitamin D hormone analogs on muscles and the reduction of falls should be of interest in a combination therapy, and could improve the anti-fracture efficacy of bisphosphonates.

Clinical studies: effects of bisphosphonates and Vitamin D-hormone analogs on BMD and fractures in PMOP

A growing amount of evidence is becoming available from randomized studies concerning combination therapies of bisphosphonates and Vitamin D-hormone analogs. Their main characteristics are summarized in Table 1.

In a prospective, randomized, controlled study, 120 women with osteoporosis were treated for 24 months with calcium (500 mg/day), alendronate (10 mg/day), calcitriol (0.5 μg/day) or with a combined treatment of alendronate (10 mg/day) + calcitriol (0.5 μg/day)[53]. The baseline characteristics of the four treatment groups were similar. BMD was measured by a total body dual-energy X-ray absorptiometry as total body mass and at different sites of interest, including the spine, trunk, arms, legs and pelvis.

BMD was significantly higher in the group of patients receiving combined therapy, as compared with the groups treated with alendronate, calcitriol or calcium. The increase in BMD of the combination, ranging from 2% to 5% yearly depending on the measurement site, was stable during the trial period and did not tend to decrease over time. Response rates of 80%, 50% and 20% were observed in the combination group, alendronate group and calcitriol group, respectively.

The efficacy of a combined treatment of alendronate and alfacalcidol has also been evaluated in a randomized open study, including 226 postmenopausal women with osteoporosis[54]. The patients were divided into two groups: Group A with 114 patients receiving alendronate 10 mg plus 500 mg calcium daily, and Group B with 114 patients receiving alendronate 10 mg plus alfacalcidol 0.5 μg/day for 12 months. After one year and as compared to baseline, the BMD of group A increased by 3.5% at LS and 2.5% at FN. The BMD of group B was increased by 4.3% at LS and 3.3% at FN. Between-group comparisons led to a statistical difference at the 5% level, suggesting that the combined treatment was significantly more effective on BMD of the most important skeletal sites than monotherapies.

A similar efficacy of alendronate and alfacalcidol was reported by a prospective, randomized, single-blind study with patients who had established postmenopausal or male osteoporosis[55]. Ninety patients (57 women, 33 men) averaging 66 years of age were randomly assigned to receive either 1 μg alfacalcidol daily+70 mg alendronate weekly+500 mg calcium daily (Group A, n=30), or 1 μg alfacalcidol+500 mg calcium daily (Group B, n=30), or 70 mg alendronate weekly+1000 mg calcium+1000 IU Vitamin D daily (Group C, n=30). Falls, fractures and back pain were assessed. During the 2-year study a significant increase of 9.6% in BMD at LS in Group A, 3.0% in Group B and 5.4% in Group C versus baseline was observed ($p<0.001$ for inter-treatment difference A versus B, C). A significant increase of 3.8% in BMD was observed at FN in Group A versus baseline, 1.5% in Group B and of 2.4% in Group C. The 2-year rates of patients with at least one vertebral fracture were 1 in Group A, 5 in Group B and 4 in Group C. The 2-year rates of patients with at least one non-vertebral fracture were 1 in Group A, 4 in Group B and 6 in Group C. Considering all fracture types together, combined therapy evidenced a significant superiority as compared to alfacalcidol monotherapy, or a combined treatment with alendronate and native Vitamin D. A significantly lower rate of falls was also documented as a result of using the combined treatment of alendronate plus alfacalcidol. After 24 months, 80% of the patients in the combination group were free from back pain, compared to 43% in the alfacalcidol group and 30% in the alendronate+plain Vitamin D group ($p<0.003$).

A prospective, multiarms, randomized, single-blind study with 197 postmenopausal osteoporotic patients was performed[56]. The patients received an oral daily dose of 0.5 μg alfacalcidol plus 10 mg alendronate (Group A), 0.5 μg alfacalcidol (Group B), 10 mg alendronate (Group C) and 500 mg calcium as the control group (Group D) once daily during 24 months. In addition, all patients in groups A, B and C received 500 mg of calcium. BMD was measured using DEXA at the beginning and after 12 and 24 months. The percentage changes of BMD at the lumbar and femoral neck levels were 6.4 and 5.0 in Group A, 1.7 and 1.2 in Group B, 4.8 and 3.6 in Group C, -1.4 and -6.0 in Group D. The BMD change differences observed in the combined therapy Group (A), as compared with alfacalcidol (Group B), alendronate (Group C) and control (Group D), were all significant ($p<0.05$). No case of hypercalcuria or of hypercalcemia was recorded.

A trial using alendronate and calcitriol showed that a 9-month administration of alendronate (5 mg daily) and a low dose of calcitriol (0.125 μg daily) was equivalent to alendronate (5 mg daily)+calcium (500 mg/daily) on BMD and bone metabolism markers[57]. These results outlined the necessity to work with doses comprised of between 5 and 10 mg of alendronate and 0.5 μg calcitriol or 0.5 μg - 1.0 μg alfacalcidol in order to derive the clinical benefits from the use of combination therapy.

D-hormone analogs have also been tested with etidronate[58-60]. Postmenopausal women with at least one non-traumatic vertebral fracture or low BMD were randomly allocated to a cyclic 400 mg/d etidronate treatment or a combination treatment (cyclic etidronate therapy plus oral 0.5 μg calcitriol daily)[58]. The mean statistically significant increase in lumbar spine BMD was 5.2% in the combination group and 2.7% in the etidronate monotherapy group after one year's treatment ($p<0.05$). The femoral neck BMD was increased by 2% in the combination group, which is significantly different from the -0.4% decrease in the etidronate monotherapy group. Similar conclusions were reached by the two other studies in this field[59,60].

On the basis of the current available clinical data, combination therapies with bisphosphonates (mainly alendronate) and Vitamin D-hormone analogs (mainly alfacalcidol) seem to be more effective in preventing bone loss in PMOP, as compared to the respective monotherapies. Due to limited statistical power, the available pool of data did not allow the detection of differences in fracture rates. Such major clinical trials already exist for bisphosphonates, including alendronate, and have been highly supportive of their commercial success[61,62]. However, meta-analyses of randomized clinical studies have revealed significant reductions of vertebral and non-vertebral fractures for Vitamin D-hormone analogs[7,8,63]. A recent summary of existing meta-analyses has suggested that the number needed to treat in order to avoid a fracture (NNT) after two years, was in the same range of order when using Vitamin D-hormone analogs (NNT=94) in comparison to other anti-osteoporotics, e.g., alendronate (NNT=72), risedronate (NNT=96) or raloxifene (NNT=99)[64]. However, these data require confirmation by large scale, head-to-head studies and further meta-analyses.

Effect of Vitamin D-hormone analogs and bisphosphonates on BMD and fractures in GIOP

It is well established that glucocorticoids (GCs) negatively affect bone through multiple mechanism pathways. Pro-inflammatory cytokines (e.g., IL-1, IL-6, IL-12, TNF-α) induce bone resorption, reduce bone formation and induce

Author #ref	Subjects	Duration (months)	Treatments	Main results (Intergroup differences, for each criterion)
Ones 56	197 PMOP	24	A) Alen 10mg+Alfa 0,5µg+Ca 500mg B) Alfa 0,5µg+Ca 500mg C) Alen 10mg+Ca 500mg D) Ca 500mg	BMD LS:A>B,C,D BMD FN:A>B,C,D Osteocalcin: NS Deoxypyr: A,B,C<D Urinary Ca: NS
Frediani 53	120 PMOP	24	A) Alen 10mg+Calci 0,5µg B) Alen 10mg C) Calci 0,5µg D) Ca 500 mg	BMD total : A>B,C,D Urinary Ca: A,D,NS C>A,B,D B<A,C,D BALP: A, C, D NS B<A,C,D Hydroxyproline: NS
Kataxaki 54	226 PMOP	12	A) Alen 10mg+Ca 500mg B) Alen 10mg+Alfa 0,5µg	BMD LS: B>A BMD FN: B>A
Ringe 55	90 PMOP MOPB)	24	A) Alfa 1µg+Ca 500mg B) Alen 70mg (weekly)+Ca 1g+Vit D 1000IU C) Alen 70mg (weekly)+Alfa 1µg+Ca 500mg	BMD LS: C>A, B BMD FN: C>A, B All Fx: C<A, B Falls: C<B
Malavolta 57	152 PMOE	9	A) Alen 10mg+Ca 500mg B) Alen 10mg+Calci 0,125µg C) Ca 500mg	BMD LS: NS BMD FN: NS Urin Ca: NS BALP: NS Hydroxyproline; NS
Masud 58	58 PMOP	12	A) Eti 400mg*+Ca 500mg B) Eti 400mg*+Calci 0,50µg+Ca 500mg	BMD LS: B>A BMD FN: B>A
Iwamoto 59	40 PMOP	12	A) Eti 200mg* B) Eti 200mg*+Alfa 1µg	BMD LS: NS Urinary NTX: B<A Back pain: NS Vert Fx: NS
Shiota 60	40 PMOP back pain	24	A) Alfa 0,5µg+Ca 2g B) Eti 200mg**+Ca 2000mg+Alfa0,5 µg	BMD LS: B>A Vert Fx: B<A

BMD: bone mineral density; PMOP: postmenopausal osteoporosis; PMOE: postmenopausal osteopenia; MOP: male osteoporosis; Eti: etidronate; Alen: Alendronate; Alfa: Alfacalcidol; Ca: Calcium; Calci: calcitriol *Daily oral Etidronate daily for 2 weeks every 3 months; ** Daily oral Etidronate for 2 weeks followed by 2,000mg Ca and 0.5µg Alfacalcidol for the next 10 weeks

Table 1. Characteristics and main outcomes of randomized controlled trials on combined therapies with bisphosphonates and D-hormone analogs in primary osteoporosis.

muscle dissipation[20,65]. The incidences of falls and non-vertebral fractures increase rapidly after the first 3 months, and revert sharply towards baseline levels after discontinuation of oral GC treatment[2].

Alendronate has been approved for the prevention and treatment of GIOP, and is fully accepted worldwide as a first line drug in this setting[66,67]. Alfacalcidol has been shown to maintain bone mass in patients taking high doses of GCs[68,69], and can in comparison to plain Vitamin D significantly reduce vertebral fracture rate[70]. Recent data tends to support the use and outline of the need for confirmatory trials of Vitamin D hormone analogs with and against some bisphosphonates in GIOP[70,71]. From a physiological point of view, *in vitro* and preliminary studies suggest that alfacalcidol could lead to a stabilization of calcium absorption, PTH levels[72], calcitriol[73-75], VDR gene expression[76,77], pro-inflammatory cytokines[21,22], and could downregulate specific T-cell immunomodulating properties which will prevent inflammation-induced loss of bone strength and muscle power[19,20,78]. Recent evidence suggests that deleterious pharmacological effects of GCs may be counteracted by using Vitamin D hormone analogs[22,68,70,79]. Recent data suggest that Vitamin D-hormones may have a synergistic immunomodulatory effect when combined with routine therapy for immunosuppression, reducing the doses of potent, but

toxic and expensive cyclosporine and glucocorticoids required to prevent organ rejection without any detectable change in episodes of rejection, infection or deaths[80]. As for PMOP, another important outcome would be the possibility for such a combination to reduce the risk of falls in patients with GIOP[2].

The clinical efficacy of different treatment schemes, including combination therapy, has been studied in several clinical trials. Forty patients with rheumatoid arthritis treated with glucocorticoids were enrolled in an open, controlled 12-month study to evaluate the effect on BMD of alendronate 10 mg per day (n=20) vs. combined treatment of alendronate 10 mg per day plus calcitriol 0.5 μg per day (n=20)[81]. Patients were divided into 2 sub-groups according to the daily dosage of prednisone (A:<8 mg; B:>8 mg). Both treatments significantly increased BMD after 12 months. The increases of BMD were significantly higher in the group receiving the combined therapy than in the group treated with alendronate alone, particularly in patients treated with higher doses of prednisone (lumbar spine L2-L4: 2.2% vs. 1.2%; femoral neck: 2.1% vs. 1.2%; total body: 1.3% vs. 0.2%; $p<0.05$).

A one-year double-blind trial compared 149 patients (122 women, 27 men), after cardiac transplantation, who were randomized to either alendronate 10 mg daily or calcitriol 0.5 μg daily versus 27 control subjects[72]. The change in spinal BMD was -0.7% with alendronate, -1.6% with calcitriol and -3.2% in controls. Among the patients in the calcitriol group who adhered to the therapy, the BMD decreased only by 0.5%. The change in femoral neck BMD was -1.7% with alendronate, -2.1% with calcitriol and -6.2% in controls. Urinary NTX fell by 34% with alendronate, 26% with calcitriol, but was unchanged with controls. By six months, the serum parathyroid hormone level had decreased in the calcitriol group and had increased in the alendronate group. New vertebral fractures occurred in 6.8% of the alendronate subjects, 3.6% of the subjects treated with calcitriol, and 13.6% of the subjects in controls (between-group difference NS).

A pilot study has shown that subjects with heart transplants receiving attendant glucocorticoid/cyclosporine therapies and treated with bisphosphonates and D-hormone (Group A) analogs sustained significantly less bone loss and significantly fewer fractures than those treated with calcium and Vitamin D (Group B). At 12 months after transplantation, there was virtually no lumbar spine bone loss in Group A patients, whereas lumbar spine BMD had declined significantly in Group B patients (0.2% +/-0.9% vs. 6.8% +/-1.0%, respectively; $p<0.0001$). Similarly, femoral neck BMD fell by 10.6% +/-1.1% in Group B patients, and by only 2.7% +/-1.4% in Group A patients ($p<.0001$). Three vertebral fracture incidences occurred in two Group A patients, whereas 17 Group B patients sustained 30 incidences of vertebral fractures[75]. It was also stated that the combination therapy using bisphosphonates and Vitamin D-hormone analogs might provide further benefits in reducing the levels of bone resorption markers after kidney transplants, as compared to monotherapy with calcitriol[82]. Patients with kidney transplants were randomized to 10 mg/day of alendronate, 0.5 μg/day of calcitriol, and 500 mg/day of calcium carbonate (Group A), or 0.5 μg/day of calcitriol and 500 mg/day of calcium carbonate (Group B). After one year, bone turnover markers showed an insignificant drop in Group B patients, while both b-ALP and u-NTX decreased significantly in Group A. Bone density of the spine (+5.0 +/-4.4%), femoral neck (+4.5 +/-4.9%), and total femur (+3.9 +/-2.8%) increased significantly only in Group A. However, no trend toward further bone loss was noticed in subjects treated with only calcitriol and calcium.

In summary, in light of the limited currently available evidence, the combination of alendronate and Vitamin D-hormone analogs remains promising in preventing bone loss in patients with high doses of GCs and/or uncontrolled activity of the basic diseases, or after organ transplants. However, more long-term high-quality factorial-designed clinical trials are required before such an approach could be suggested as a routine practice.

Relative efficacies of combined therapies involving bisphosphonates, alfacalcidol, calcitriol and native Vitamin D

Vitamin D and calcium supplementation are required to balance frequent Vitamin D deficiency after menopause while their use with bisphosphonates is required to avoid hypocalcemia. In this perspective, Vitamin D should be regarded as a basic nutritional supplement and not as a pharmacological combination therapy. However, Vitamin D-hormone analogs, including alfacalcidol, have demonstrated their synergistic efficacy when combined with bisphosphonates in Vitamin D replete patients, which in turn puts this approach into a new therapeutic slot.

In a pre-clinical trial the respective efficacies of alfacalcidol and plain Vitamin D on bone quality was investigated[27]. The mechanical bone strength at the femur of 8-month-old ovariectomized rats was analyzed with a three-point bending test, and at the vertebral body specimens a compression test was used. In that study alfacalcidol increased BMD and bone strength more effectively than plain Vitamin D did while allowing for similar levels of effect on calcium absorption.

A recently published meta-analysis that studied the efficacy of plain Vitamin D combined with Vitamin D-hormone analogs on falls in the elderly, supports the effect of "Vitamin D" as compared to calcium and placebo[83]. Based on 5 prospective, randomized controlled clinical studies a 22% reduction of the Odd's ratio (OR) for fall rate was calculated (OR 0.78; 95% Cl 0.64-0.92). A pre-planned sub-group analysis was performed to compare the effect-sizes of plain Vitamin D and Vitamin D-hormone analogs. For 3 studies involving 613 participants treated with Cholecalciferol, the OR for falling was 0.83 (95% CI 0.65-1.06). In contrast the OR for the two-pooled studies involving 626 patients treated with Vitamin D-hormone analogs was significant (OR 0.71, 95% Cl 0.55-0.92). However, it is worthwhile to mention that most participants in the plain Vitamin D group were Vitamin D-deficient, as opposed to the participants treated with Vitamin D-hormone

analogs who had normal serum Vitamin D levels, which limits the comparability of treatment regimens.

The results of studies with plain Vitamin D with or without calcium outline that a significant number of the elderly with or without osteoporosis, even with normal Vitamin D serum levels (>10-20 ng/ml), are based on a lower CrCl (<65 ml/min) not able to synthesize the active hormone. In this context, it is of importance to note that serum levels of calcitriol, but not of Calcidiol, have been found to be significantly correlated with cognitive function in elderly women, given that reduced cognitive capabilities are independent risk factors for falls[84]. The observed beneficial effect of Vitamin D-hormone analogs on the observed reduction of falls and fractures can, at least partially, be explained by the expression of its own receptor and a hormone-like effect on bones, muscles and the brain.

The individual and respective efficacies of native Vitamin D and Vitamin D-hormone analogs in osteoporosis are highly debated. The effects of Vitamin D and calcium are evident in patients with Vitamin D deficiency, while the evidence supporting the efficacy of that treatment in Vitamin D replete subjects remains limited and is currently being highly controversial. In two recent randomized, double-blind, placebo-controlled studies in elderly people aged 80 and above it was shown that a single oral dose or an intramuscular administration of 300,000 IU plain Vitamin D over a period of 6 months and three years failed to reduce the fall rate and the risk of hip and non-vertebral fractures[85,86]. Another randomized controlled trial of orally administered Vitamin D (800 IU daily) in combination with calcium (1 g daily) to 3,322 women over 70 with at least one risk factor for hip fracture after 25 months of treatment evidenced no significant difference between the supplemented group and the non-supplemented group concerning falls and hip fractures[87]. The findings of the latest randomized placebo-controlled trial of daily oral Vitamin D (800 IU) and/or calcium (1 g) in 5,292 women (85%) and men aged 70+ with established osteoporosis followed-up for 24 and 62 months, also failed to demonstrate a significant superiority of supplementation with calcium, plain Vitamin D or their combination for the prevention of falls and osteoporotic fractures[88].

A recent comparative meta-analysis confirmed that alfacalcidol or calcitriol exhibited better efficacy in preventing vertebral bone loss, and vertebral and non-vertebral fractures as compared to plain Vitamin D in postmenopausal osteoporosis[8]. Results were expressed as effect-sizes for bone loss and as rate difference (RD) for fracture while allocated to active treatment or control. With respect to BMD, Vitamin D-hormone analogs versus placebo studies had an ES of 0.36 ($p<0.0001$), whereas plain Vitamin D versus placebo had an ES of 0.17 ($p=0.0005$), the interclass difference being highly significant (ANOVA-1; $p<0.05$). When comparing the adjusted global relative risks for fractures when allocated to Vitamin D-hormone analogs or plain Vitamin D, alfacalcidol and calcitriol provided a more marked preventive efficacy against fractures: RD=10% (95% CI, 2 to 17) compared to RD=2% (95% CI, 1-3), respectively, with significant interclass difference (ANOVA-1, $p<0.0001$). The analysis of the vertebral and non-vertebral fracture rates showed that fracture rates differed between the two classes in favour of Vitamin D-hormone analogs, with significant 13.4% risk difference (95% CI, 7.7 to 19.8,) and 6% risk difference 95% CI, 1 to 12), respectively.

Tolerance of the combination of bisphosphonates + Vitamin D-hormone analogs

In three animal trials investigating the efficacy and safety of the combination of alendronate and alfacalcidol[30], risedronate and calcitriol[29] or etidronate and alfacalcidol[38], it was shown that the co-administration of a bisphosphonate limited the risk of hypercalcemia, even when using supraphysiological doses of the Vitamin D-hormone analogs[29,38]. This finding has been confirmed in experimental autoimmune encephalomyelitis, where higher doses of a Vitamin D-hormone analog could be used in combination with alendronate without increased hypercalcemia[89].

Several preliminary comparative, factorial-designed, studies assessed the safety profile of alendronate alone, or in conjunction with calcitriol or alfacalcidol[53,56]. Frediani et al.[53] reported safety data. Serum calcium did not show any significant variation between or among the four treatment groups (500 mg calcium, 10 mg alendronate, 0.5 μg calcitriol or 10 mg alendronate plus 0.5 μg calcitriol daily) during the two years of treatment. Twenty-four hour urinary levels of calcium were significantly increased after 3 months of therapy with calcitriol, were significantly reduced with alendronate and remained stable in the combined therapy group. Urinary hydroxyproline decreased significantly in patients treated with alendronate, and in those treated with alendronate plus calcitriol no significant differences were observed between the two groups. The increased urinary calcium concentrations induced by calcitriol appeared to be related to an increased intestinal absorption of calcium and not to accelerated osteoclastic re-absorption, as documented by the constant values of hydroxyproline and by the stability of the BMD values. After 1 to 3 months, patients treated with alendronate showed a significant reduction in urinary calcium concentrations, reflecting its anti-osteoclastic effect. In combined therapy, the stability of urinary calcium, compared with basal values, could be explained by the balance between the reduction of the osteoclastic re-absorption and increased intestinal absorption of calcium. Overall, there were 13 cases of gastric pain: 8 cases in the group treated with calcium, 3 cases in the group treated with alendronate + calcitriol, and 2 cases in the group receiving alendronate (NS). Ones and Schacht also observed in their study of a combined treatment of alfacalcidol and alendronate no case of hypercalcuria or hypercalcemia[56].

In summary, current data tend to show that the episodes of hypercalcuria induced by Vitamin D-hormone analogs seem to be limited when a combined therapy with a bisphosphonate is established. There is no hint that the known side effect pattern of alendronate, especially the gastrointestinal side effects, may be changed by concomitant administration of Vitamin D-hormone analogs[53,56]. On the other side the debated long-term side effects of bisphosphonates, e.g., a reduced mineralization and

especially the decreased coupled bone remodeling with the consequences of reduced micro- and macro-fracture healing and decreased bone quality ("frozen bone") might be reduced by intermittent or continuous co-administration of Vitamin D-hormone analogs on the basis of pre-clinical studies[28-30,37]. As for clinical aspects of such an innovative approach, further long-term, confirmatory trials are required to clearly demonstrate and assess the risk/benefit profile of monotherapies as compared to combination therapies in various target patients.

Conclusion

The basis of a future optimal therapy for the prevention of fractures is to increase bone mass and strength, as well as reducing falls in the long-term without exposing the patient to serious deleterious effects. Pre-clinical and clinical data featuring bone metabolism markers, bone mineral density and fracture data suggest that combination therapies of Vitamin D-hormone analogs with bisphosphonates, e.g., alendronate and alfacalcidol, might warrant better absolute and relative efficacies in preventing bone loss and fractures due to their two different and complementary modes of action. Synergy may lie in the fact that the data strongly suggests that, apart from their pleiotropic effects on the musculoskeletal, immune and neurological systems, Vitamin D-hormone analogs are able to restore a better osteoblastic/osteoclastic balance via distinct mechanisms. Furthermore, in indirect and direct head-to-head comparative settings, D-hormone analogs have been proven to be more effective than native Vitamin D in preventing bone loss and fractures, which strengthens the claim that such an approach should not be disregarded as a very valuable alternative in the management of osteoporosis.

Negative interactions between both drugs have not yet been reported, while a reduction of hypercalcuria has been noted in combination therapies.

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