REVIEW

A Rationale for an Approach to Preventing and Treating Bisphosphonate-related Osteonecrosis of the Jaw With Vitamin K_2 Menaquinone-4

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Abstract

In this article, the authors describe the Neustadt-Pieczenik Collagen Damage and Restoration Hypothesis, which proposes that medical professionals may prevent and treat osteoporosis and bisphosphonate-related osteonecrosis of the jaw successfully by protecting bone collagen from damage and by stimulating production of new bone collagen. They posit that collagen damage represents the keystone for understanding these diseases. The article discusses studies confirming that collagen production is critical to bone health, fracture healing, and fracture prevention and that MK4, a specific form of vitamin K_2, is important for its ability to stimulate collagen production.

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Dental professionals have defined osteonecrosis of the jaw (ONJ) as "exposed bone in the mandible, maxilla, or both that persists for at least 8 weeks, in the absence of previous radiation and of metastases in the jaws."[1,2] ONJ caused by bisphosphonates (BPs: brand names, Fosamax, Zometa, Aredia, Actonel, Boniva), called bisphosphonate-related ONJ (BRONJ), is an iatrogenic disease of significant concern to patients and dentists. In 2003, Marx described 36 cases of BRONJ.[3] Since then, others have reported additional cases,[4-7] and many dental professionals, particularly oral and maxillofacial surgeons, have identified numerous unpublished cases.

Marx's original publication reported 36 diagnosed cases under treatment,[3] which increased to 76 cases as of 2005.[8] Among the other publications, Migliorati reported five cases,[4] Ruggerio et al reported 63 cases,[7] Carter and Gross reported four cases,[5] and Estilo et al reported 13 cases.[6] In a previous article, Marx also mentioned 43 additional cases reported to him nationwide by colleagues who had sought advice regarding prevention and management of this condition.[8]

Currently, BPs are the standard of care for millions of men and women with osteoporosis and also are used widely for patients with Paget's disease, bone malignancies, and multiple myeloma in patients with breast and prostate cancer. The rationale for using BPs in osteoporosis is that these medications increase bone mineral density (BMD) and decrease fracture risk. Some medications, such as the bisphosphonates, enhance BMD by inhibiting resorption of trabecular bone by osteoclasts. Oral BPs prescribed for osteoporosis include etidronate (Didronel), risedronate (Actonel), tiludronate (Skelid), alendronate (Fosamax), and ibandronate (Boniva). In 2006, alendronate was the most widely prescribed oral BP, accounting for 37.7% of the osteoporosis drug market and generating $2 billion in sales in the United States alone.[9]

The most potent BPs are delivered intravenously (IV) and include pamidronate (Aredia) and zoledronate (Zometa, Reclast). These BPs are indicated to stabilize metastatic cancer (primarily breast and prostate) deposits in bone, to treat the bone resorption defects of multiple myeloma, and to correct severe hypercalcemia. Additionally, the US Food and Drug Administration (FDA) approved IV zoledronate in 2007 for treatment of postmenopausal osteoporosis.

In 2007, Khosla and Burr estimated the risk of ONJ in patients with cancer treated with high doses of intravenously applied BPs to be in the range of 1% to 10%.[1] Oral BPs can also cause BRONJ. In 2009, a retrospective article concluded that the actual risk of BRONJ from oral BPs is 4%.[10] This study was the first large institutional one in the United States to evaluate the epidemiology of BRONJ. Clinicians may find it instructive to understand the risk factors for BRONJ identified in this study (Table) so that they can best manage their patients on BPs. The risk, however, may be much higher. A more recent study concluded that the risk for BRONJ from oral bisphosphonates was actually 8%, double what had been previously reported.[11]

Clinicians during their careers undoubtedly will encounter patients who are taking BP medications. Recognizing the risk factors for BRONJ, understanding its pathophysiology, and

Table. Risk Factors for Developing Bisphosphonate-related Osteonecrosis of the Jaw (BRONJ) From Oral Alendronate Therapy[10]

Age	>63 years old
Gender	Female
Duration of treatment	12 mo or longer
Common comorbidities associated with BRONJ	Type 2 diabetes, hypertension, hypercholesterolemia, steroid therapy, chemotherapy
Procedures that initiated BRONJ	Tooth extraction

enacting a rational approach to prevent it in patients, are crucial to providing safe and effective treatments.

In this article, the authors propose a rational approach for moving BRONJ away from the risk management and quality assurance model that dentists currently use to a preventive model. The approach outlined in this article admittedly requires additional study, including clinical trials, but the conceptual framework, based on a broad review of the ONJ literature, provides a way to begin to address this situation in a more proactive way.

Researchers have studied the etiology of BRONJ extensively.[12-15] Although they have not reached a consensus yet as to its underlying pathophysiology, their hypotheses include decreased angiogenesis leading to avascular necrosis,[15,16] suppression of bone turnover due to attenuated osteocyte viability and resultant microfractures,[12,17,18] fibroblast suppression,[19] immunosuppression,[20,21] decreased viability of oral keratinocytes,[22] and oral microbial biofilms migrating to exposed jawbone from dental procedures (eg, tooth extraction).[23] All of these physiological disturbances lead to decreased wound healing that could contribute to the initiation and propagation of BRONJ.[2]

While it is the authors' position that BRONJ does involve these pathological variables, they also firmly believe that collagen damage is the initiating factor in BRONJ and that all other pathophysiological mechanisms follow. Although a 2010 study by German researchers Simon et al[14] hinted at the possibility of this conclusion, until now researchers have not incorporated it into a comprehensive model for BRONJ.

THE NEUSTADT-PIECZENIK COLLAGEN DAMAGE AND RESTORATION HYPOTHESIS

The Role of Collagen

Developed largely from research on diabetes, osteoporosis, and BRONJ, the Neustadt-Pieczenik Collagen Damage and Restoration Hypothesis (CDRH) posits that collagen damage is the keystone for understanding, preventing, and treating BRONJ and osteoporosis. Collagen provides the basic functional properties of the most vulnerable tissues, such as renal basement membrane, the cardiovascular system, retinal capillaries, and the extracellular matrix component of bone.

The authors hypothesize that decreased collagen quality and/or quantity (collagen damage) results in decreased collagen strength and creates a microenvironment more susceptible to microfractures from mastication. This in turn provides a pocket for bacteria to seed and reproduce when the thin periodontal tissue is breached and bacteria migrate into the bone. Damaged collagen also decreases arterial flexibility, thereby accounting for decreased bone perfusion that creates a nutrient-poor and hypoxic environment that becomes a feed-forward system causing further tissue damage, immunosuppression, avascular necrosis, and BRONJ.

If collagen degradation itself can be prevented and reversed, BRONJ may be effectively prevented and more effectively treated. This is the essence of the CDRH and its application to BRONJ.

The preeminent role that collagen plays in bone health, fracture healing, and fracture prevention is just starting to emerge in dental research, but the general medical literature on osteoporosis and bone histology describes its role well. Bone matrix is a dynamic, two-phase system in which the mineral phase provides the stiffness and the collagen-fibers phase provides the ductility and ability to absorb energy (ie, the toughness). Therefore, alterations of collagen properties can affect the mechanical properties of bone and increase fracture susceptibility.[24]

Type I collagen is the most abundant type of collagen and is widely distributed in almost all connective tissues with the exception of hyaline cartilage. It is the major protein in bone, skin, tendon, ligament, sclera, cornea, and blood vessels. The quality of this connective tissue influences bone macro- and microarchitecture. Type I collagen comprises approximately 95% of the entire collagen content of bone and about 80% of the total proteins present in bone.[25] Type I collagen molecules are linked together by different crosslinking molecules, including pyridinoline (PYD) and deoxypyridinoline (DPD) in the C-telopeptide (CTX) and N-telopeptide.[26]

In healthy bone, collagen is continuously renewed. Following secretion from the cell, collagen undergoes numerous posttranslational modifications and is eventually stabilized by intra- and intermolecular crosslinks formed through both enzymatic and nonenzymatic processes.[24] PYD and deoxypyridinoline DPD are produced by enzymatic crosslinking and are generally indicative of mature collagen.[24] As mean tissue age increases, collagen undergoes isomerization reactions, altering the structure of the collagen molecule.[24,27,28] Quantifying the ratio of isomerized (β) to native (α) collagen as βC-telopeptide (βCTX) to αCTX, respectively, provides an index of collagen maturity.[26]

Pentosidine (PEN) is produced by nonenzymatic glycation and is generally higher in bone having a greater mean tissue age.[24] PEN is an advanced glycation end product (AGE), which results from the reaction of carbohydrates in the extracellular matrix with amino groups on cumulative damage to proteins by AGEs.[29,30]

AGEs alter the structural properties of tissue proteins and reduce their susceptibility to catabolism.[31,32] These changes contribute to the aging of tissues and, when accelerated by hyperglycemia as in diabetes and metabolic syndrome, to the gradual development of tissue-specific and disease-specific complications. AGEs, and specifically PEN, not only increase with age,[33,34] in diabetes mellitus,[35,36] and in renal insufficiency,[37-39] but they also play an important role in increasing stiffness of articular cartilage[40] and in contributing to arteriosclerosis and hypertension by decreasing arterial elasticity.[41] AGEs are also involved in the pathogenesis of

diabetic complications,[35,42-45] atherosclerosis,[42,45-47] rheumatoid arthritis,[48-50] and impaired angiogenesis.[51] Recently elevated and increasing serum PEN was shown[52] to indicate a poor prognosis and a worsening of patients' conditions in ischemic stroke and to be a prognostic factor in heart failure.[53] AGEs also were shown[54] to decrease fracture healing by up to 63% in a rat model of diabetes.

The propensity of individual trabeculae to fracture (microfracture) when a whole bone is overloaded but not overtly fractured is important for understanding the multiscale failure mechanics of bone. Fractured trabeculae can weaken bone by diminishing the load-carrying capacity of the cancellous structure.[55] Because resorbed trabeculae may not be replaced during tissue repair, a microfracture may have a permanent detrimental effect on bone volume fraction, microarchitecture, and the mechanical properties of the cancellous structure.[56,57]

Whether or not a loaded trabecula fractures is determined in part by its ductility,[57] a failure property that describes brittleness independent of strength. Two structures may have the same strength—ie, they may both be able to withstand the same magnitude of force—but one may easily fracture like chalk while another may be very ductile like rubber. In cadaver lumbar vertebrae[57] and femora,[58] PEN was associated with up to 35% decrease in ductility. Thus elevated PEN increased bone's susceptibility to microfracture.

In addition to decreasing bone ductility, PEN has been shown to increase bone stiffness as indicated by decreased bending and compressive yield stress. Studies on in vitro aging of fetal bovine cortical bone[59] demonstrated that the amount of PEN was associated with a 30% decrease in bending and compressive yield stress and a 2.5-fold increase in compressive post-yield energy absorption. In another study of human lumbar vertebrae,[60] the amount of PEN was shown to be related to the biomechanical properties of bone independent of BMD. AGEs have been associated with a stiffer collagen network that may become more easily fatigued and brittle40; a decrease in the mechanical integrity of cortical and trabecular bone[57,58,61]; and an increase in skeletal fragility and microfractures.[57,58,61]

PEN has consistently been implicated in increasing bone fragility and fracture risk in osteoporosis. With aging, PEN and other AGEs accumulate in osteoporotic bone and other tissues.[29,60] PEN causes altered bone remodeling in osteoporosis, and patients with osteoporosis have significantly elevated serum PEN compared to age-matched controls.[62] Most significantly, perhaps because of its immediate clinical relevance, a study of 423 postmenopausal osteoporotic women revealed urinary PEN to be a significant predictor of vertebral fracture (odds ration, 1.33; 95% confidence interval, 1.01-1.76, $P = .04$).[63]

Bisphosphonates Damage Collagen

An in vivo study by Allen et al[64] in mature female beagles showed that BPs damage bone collagen. Dogs were treated for 1 year with oral control solution (1 mL/kg/d saline), risedronate sodium (0.05, 0.10, or 0.50 mg/kg/d), or alendronate sodium (0.10, 0.20, or 1.00 mg/kg/d). The middle doses of risedronate (0.10 mg/kg) and alendronate (0.20 mg/kg) correspond to treatment doses for postmenopausal osteoporosis on a mg-per-kg basis. Collagen quality was determined by measuring levels of PYD, DPD, and PEN crosslinks in lyophilized bone powder. Collagen quality was significantly reduced compared to control for bisphosphonates in a dose-dependent manner.

At all doses, both risedronate- and alendronate-treated animals had significantly higher concentrations of PEN in the vertebral trabecular bone matrix compared to vehicle-treated animals. For risedronate, levels of pentosidine were +36% (0.05 mg/kg), +50% (0.10 mg/kg), and +58% (0.50 mg/kg) higher than control (all $P < .05$). The highest risedronate dose had significantly higher PEN concentrations compared to the lowest risedronate dose. For alendronate, levels of PEN were +34% (0.10 mg/kg), +37% (0.20 mg/kg), and +52% (1.00 mg/kg) higher than control (all $P < .05$); the highest dose had significantly higher concentrations of PEN compared to the lowest dose. There was no significant difference in PEN levels between risedronate and alendronate at any of the three dose-equivalents.

The ratio of PYD to DPD was significantly higher for all doses of both risedronate and alendronate compared to control. The ratio of PYD/DPD was +20% to +24% in risedronate-treated animals compared to control, with no difference among the three doses. Alendronate-treated animals had a +14% to +26% higher PYD/DPD ratio compared to control with no difference among the three doses. There was no difference between risedronate and alendronate at any of the dose-equivalents.

Changes in the ratio of PYD/DPD in the risedronate- and alendronate-treated groups were the result of lower DPD levels. All doses of alendronate resulted in significantly lower DPD compared to control, while risedronate showed a trend toward lower DPD levels ($P = .057$) compared to control. There was no change in PYD between control and any of the treatment groups ($P = .21$ to $.79$).

Similar results have also been demonstrated by Byrjalsen et al[65] in 27 postmenopausal women taking either oral daily or intermittent BPs. The researchers evaluated the effects of oral BPs on collagen quality in women taking 10 mg alendronate daily (n = 14), 20 mg alendronate daily (n = 13), 2.5 mg ibandronate daily (n = 36), 20 mg ibandronate every second day for 24 days every 3 months (n = 36), or placebo (n = 39). Collagen quality was measured as a ratio of urinary bone collagen fragments (αCTX-to-βCTX); αCTX represents newer, younger collagen, and βCTX reflects older, more mature collagen. After 6 months of oral BP, large differences were demonstrated, with the bisphosphonate group representing an older collagen. After 6 months of treatment, the ratio in the alendronate group was decreased by 38% and progressively decreased with 50% at 12 months and 61% at 24 months of treatment. In the ibandronate group, the ratio was decreased with 40% at 6 months of treatment and remained decreased with 36% at 12 months of treatment. The placebo-corrected changes in the ratio were highly statistically significant with a time-averaged reduction in the alendronate group of 52% during the 2-year treatment period ($P < .001$) and a 38% reduction in the ibandronate group during 12 months of treatment ($P < .001$).

Simon et al investigated the effects in vitro of 28 days of zoledronate and pamidronate exposure on collagen production in gingival fibroblasts, osteoblasts, and human osteogenic osteosar-

coma cells (SaOS-2).[14] To relate the scientific research to clinical processes better, the researchers used the most common drugs associated with ONJ: zoledronic acid and pamidronate.

They isolated human osteoblasts and fibroblasts from iliac crest and gingiva of healthy patients who were not taking BP medications. They used untreated cells as the control group and inorganic pyrophosphate (PP) as a positive control. They cultured cells for 28 days in growth medium, with each cell line cultured in 10 groups of six well plates. Every third day, they renewed the growth medium with zoledronic acid, pamidronate, and PP at four different concentrations each: 1 µM, 5 µM, 10 µM, and 20 µM. They evaluated gene expression by real-time reverse transcription polymerase chain reaction after reverse transcription of the corresponding mRNA and analyzed collagen expression by enzyme-linked immunosorbent assay.

At higher than 1 µM, zoledronic acid and pamidronate were toxic to cells; higher concentrations yielded insufficient gene expression and collagen production for analyses. At all concentrations used, zolendronate reduced gene expression to <16%. Thus gene amplification was possible only for cells cultured in 1 µM pamidronate, which did not suppress gene expression as much as zoledronic acid. Pamidronate reduced gene expression significantly compared to the control ($P < .05$). Fibroblasts showed a maximum of 31% gene expression, osteoblasts 56%, and SaOS-2 14%. The mean expression of type I collagen production significantly decreased in all cell lines ($P < .05$) compared to control by treatment with 1 µM each zolendronic acid and pamidronate.

This study demonstrates for the first time that zolendronic acid and pamidronate are directly toxic to osteoblasts, fibroblasts, and SaOS-2 cells and significantly negatively affect collagen production. The reduction in extracellular matrix production of these cell lines after BP exposure could possibly explain why patients experience BRONJ and decreased wound healing. It is unclear, however, why osteonecrosis is mainly found in the jaws and not other bones in these patients. Since many factors can influence the clinical outcomes and negative sequelae of BP therapy, further investigation needs to be pursued to verify these findings, test other BPs such as alendronate and ibandronate, and expand the clinical implications of these data. Notwithstanding the limitations of this study, type I collagen was reduced in all 3 cell lines treated with zoledronate and pamidronate, which provides compelling in vitro evidence for the possible in vivo effects of the collagen-destroying potential of BPs.

Decreased bone collagen production as indicated by bone resorption markers leads to increased susceptibility to fractures and osteoporosis. In prospective epidemiologic studies, researchers have shown that markers of bone turnover, particularly markers of bone resorption, are associated with fracture risk,[66-71] and this association appears to be independent of BMD.[72]

The limitations of BMD in predicting fracture risk are well documented in multiple studies. In 1996, researchers concluded in a meta-analysis of prospective cohort studies, totaling about 90 000 person-years of observation time and more than 2000 fractures, that BMD predicts less than 50% of patients who progress to fractures.[73] Another study published in 2004 that analyzed data from 7806 men and women from the Rotterdam Study—a prospective, population-based cohort study of men and women aged 55 years and older—concluded that bone density scans predict only 44% of women and 21% of men aged 65 years and older who progress to a nonvertebral fracture.[74]

In a larger meta-analysis published in 2004 of 15 259 men and 44 902 women from 13 different cohorts and totaling 250 000 person-years, researchers evaluated the 10-year risk of a fragility fracture in postmenopausal women with osteoporosis (T scores of –2.5 or less).[75] They concluded that in men and women, bone density scans predict only 22% of hip fractures. More recently in 2006, the North American Menopause Society published a position statement on the predictive value of bone density scans in which they concluded fracture risk "depends largely on factors other than BMD."[76]

Since collagen is not detected by X-rays, it is not included in a bone density scan report. Thus, in reporting only the density of bones, the scans are evaluating bone quantity and not bone quality. Any discussion of bone quality would be incomplete without considering the pivotal role of bone collagen.

Developed largely from research on osteoporosis, the CDRH posits that collagen destruction represents the keystone for understanding, preventing, and treating osteoporosis and BRONJ. With respect to BRONJ, the authors hypothesize that collagen damage creates a microenvironment more susceptible to microfractures from mastication. In turn, these microfractures provide a pocket for bacteria to seed and reproduce. Decreased angiogenesis and immune modulation may contribute to collagen degradation and necrotizing tissue infections with a markedly impaired wound-healing capacity in BRONJ. If dental professionals can prevent and reverse collagen degradation, they may be able to prevent and treat BRONJ effectively. This concept is the essence of the CDRH and its application to BRONJ.

Why Bisphosphonate-related Osteonecrosis May Occur Only in the Jaw

Two possible reasons why bisphosphonate-related osteonecrosis affects jaws are that masticatory forces apply great pressure to the mandible and maxilla over a relatively small volume of tissue compared to forces applied to other bones during routine daily activities. The forces of chewing could thus provide ample pressure for microfractures to occur with concomitant inhibition of fracture healing by BP medications.

One study by van der Bilt et al[77] published in 2008 in the European Journal of Oral Sciences evaluated the bite force in 81 dentate volunteers (13 men and 68 women). The mean age of the men was 37 ± 16 years (range 22-62 y) and of the women 39 ± 14 years (range 19-69 y). Only healthy volunteers with natural dentition were included. Maximum vertical interocclusal bite forces were measured using a bit force transducer, which consists of unilateral or bilateral strain gauges mounted on a mouthpiece. Forces were measured with volunteers clenching as hard as possible on the right side of the jaw, on the left side of the jaw, and on both sides of the jaw simultaneously. The bilateral bite force was measured at 569 N and was about 30% larger than the unilaterally measured bite forces (430 N and 429 N for the right bite force and left bite force, respectively). This considerable force, combined with

increased bone fragility from BP, may over time create a pathological environment of microfractures.

The role of microdamage was also suggested in the 2006 Annals of Internal Medicine systematic review article by Woo et al.[78] In this review of the literature on BP and ONJ, the authors postulate that ONJ "results from marked suppression of bone metabolism that results in accumulation of physiological microdamage in the jawbones, compromising biomechanical properties."[78] We postulate in the CDRH that it is not just suppressed bone metabolism but rather the direct damage of collagen by BP that results in decreased ductility, increased collagen fragility, and microfractures. This establishes an environment that predisposes the patient to BRONJ.

The medical literature on osteoporosis clearly describes the role bone collagen plays in fracture risk. Decreased bone collagen production as indicated by bone resorption markers leads to increased susceptibility to fractures and osteoporosis. Markers of bone turnover, particularly markers of bone resorption, have been shown in prospective epidemiologic studies to be associated with fracture risk,[66-71] and this association appears to be independent of BMD.[72]

BMD alone predicts less than half of patients who will sustain fragility fractures.[73] For women aged 55 years and older, the predictive value for fractures of bone density scans is just 44% for women and 21% for men age 65 and older for nonvertebral fractures.[74] A third study concluded that the 10-year predictive value was just 22% for hip fractures in men and women.[75]

Anabolic Agents for Possible Prevention and Treatment of Bisphosphonate-related Osteonecrosis of the Jaw

Anabolic agents that build bone collagen may prove useful in a clinical strategy to prevent and treat BRONJ. There is, however, a paucity of anabolic agents that meet the criteria necessary to make them top candidates. Obviously, the optimal agent would be shown to be safe and effective. This means that in animal models and humans, the agent would promote bone collagen production, prevent fractures, speed fracture healing, and prevent and reverse bone damage caused by medications, including BPs. In addition, of course, the agent would prevent and treat BRONJ.

The agent that comes closest at this point to meeting these criteria is menatetrenone or menaquinone-4 (MK4), a form of vitamin K_2. In osteoporosis research, researchers have studied vitamin K extensively for its ability to stimulate collagen production, promote bone health, and decrease fracture risk. Vitamin K is a group of structurally similar, lipid-soluble, 2-methyl-1,4-napthoquinones that include phylloquinone (K_1), menaquinones (K_2), and menadione (K_3).[79] Plants synthesize vitamin K_1, and bacteria can produce a range of vitamin K_2 forms, including the conversion of K_1 to K_2 via bacteria in the small intestines. Vitamin K_3 is synthetic, and because of its toxicity, the FDA has banned it for use in dietary supplements. It remains in limited use as an antineoplastic agent. In contrast to vitamin K_3, no known toxicity exists for the vitamin K_1 and K_2 forms.

Two major forms of vitamin K_2 exist: MK4 and menaquionone-7 (MK7). Among the vitamin K analogues, the form most researched for osteoporosis treatment and bone health is MK4. The body converts K_1—in testes, pancreas, and arterial walls—into MK4.[80] While major questions still surround the biochemical pathway for the transformation of K_1 to MK4, studies have demonstrated that the conversion is not dependent on gut bacteria because it occurs in germ-free rats[81,82] and in rats parenterally administered K_1.[83,84] In fact, tissues that accumulate high amounts of MK4 have a remarkable capacity to convert up to 90% of the available K_1 into MK4.[81,82] Recently, researchers have shown that K_1 is converted in vitro to MK4 in the MG-63 human osteoblastic cell line[85] and that in humans, the UbiA prenyltransferase containing 1 enzyme is responsible for conversion of K_1 to MK4.[86]

In contrast to MK4, humans do not synthesize MK7; instead, gut microbiota convert it from phylloquinone in the intestines.[87] Bacteria-derived menaquinones, however, appear to contribute minimally to overall vitamin K status.[88,89] Dietary supplements for bone health in the United States include both MK4 and MK7. The FDA has not approved any form of vitamin K for the prevention or treatment of osteoporosis; however, MK4 and MK7 beneficially affect surrogate markers of bone quality and health.

Researchers have studied extensively the molecular mechanisms for the beneficial effects of vitamin K on bone health and other conditions. The family of K vitamins—including phylloquinone, MK4, and MK7—are fat-soluble vitamins that act as coenzymes for a vitamin K–dependent carboxylase enzyme that catalyzes carboxylation of glutamic acid, an amino acid, resulting in its conversion to gamma-carboxyglutamic acid (Gla). This carboxylation reaction is essential for formation of bone collagen, which allows bone to deform upon impact without fracturing: eg, during mastication or a fall. Although vitamin K–dependent gamma-carboxylation occurs only on specific glutamic acid residues in a small number of proteins, it is critical to the calcium-binding function of those proteins.

Researchers have isolated three vitamin K–dependent proteins in bone: osteocalcin, matrix Gla protein (MGP), and protein S. Osteocalcin is a protein that osteoblasts synthesize, and the active form of vitamin D, 1,25-$(OH)_2D_3$ (also called calcitriol), regulates it. The mineral-binding capacity of osteocalcin requires vitamin K–dependent gamma-carboxylation of three glutamic acid residues. MGP helps regulate calcium deposition in bone and arteries,[90,91] and protein S forms part of organic bone matrix and is also involved in maintaining bone mineral density.[92] Elevated undcerbaxoylated osteocalcin (ucOC) is associated with increased fracture risk,[93,94] and clinical trials have shown that phylloquinoine,[95] MK4,[96-99] and MK7[100,101] all reduce ucOC.

While studies have shown that the presence of phylloquinone, MK4, and MK7 inversely correlate with ucOC and fracture risk and that all three decrease ucOC, ucOC is a surrogate marker. As such, its value is limited and may actually not explain or predict the fracture-prevention effects of vitamin K.

In fact, the traditional view that the osteoprotective effects of vitamin K are solely due to its effects on osteocalcin may not be correct or at least are incomplete. In 1996, researchers bred osteocalcin-deficient mice to compare the effect on bone histomorphology in animals that were unable to synthesize osteocalcin.[102] Compared to wild-type mice, the genetically altered, osteocalcin-

deficient mice demonstrated greater bone strength at 6 months of age without any differences in bone mineralization. This finding suggests that another pathway may mediate the skeletal benefits of vitamin K.

In addition to stimulating carboxylation of osteocalcin, vitamin K also regulates signal transduction and transcriptional functions. In vitro, MK4 and MK7 both induce osteoblast differentiation by binding to and activating the steroid and xenobiotic receptor/pregnane X receptor, which upregulates alkaline phosphatase and MGP.[103-105] Recently, researchers showed in vitro that MK4 and MK7 both inhibit nuclear factor κB, which is involved in osteoclastogenesis, function, and survival.[106]

While molecular markers are important, the most clinically relevant endpoints are (1) reduction of fractures in prospective, randomized clinical trials; (2) reduced healing time from fractures; and (3) prevention and reversal of bone loss from medications, including BPs. In this regard, only MK4 comes closest to meeting all these criteria.

In Japan, MK4 has been approved by the Ministry of Health for the prevention and treatment of osteoporosis since 1995.[107] As such, it has been extensively studied and shown[108] to decrease fractures in clinical trials up to 87% independent of the number of falls sustained. In clinical trials, MK4 (45 mg daily) prevented bone loss and/or fractures caused by corticosteroids (eg, prednisone, dexamethasone, prednisolone),[109-112] anorexia nervosa,[113] cirrhosis of the liver,[114] postmenopausal osteoporosis,[96,98,99,107,115,116] disuse from stroke,[117] Alzheimer's disease,[108] Parkinson's disease,[118] primary biliary cirrhosis,[119] and leuprolide treatment for prostate cancer.[120] Pathological fractures are a serious problem resulting from skeletal unloading in handicapped children. Sugiyama et al[121] published a case report of an institutionalized, bedridden 8-year-old girl with Arnold-Chiari deformity with low BMD whose BMD increased with MK4 treatment. MK4 also inhibited phenytoin-induced bone loss in rats[122]; prevented and increased bone formation in neurectomized rats,[123,124] an animal model for immobilization osteoporosis; prevented and increased bone formation in orchidectomized rats,[124] an animal model for secondary osteoporosis caused by testosterone deficiency; and improved healing time and bone quality in experimentally induced osteotomy in rats alone and in the presence of glucocorticoids.[125] MK4 therapy also has been cited[122] as a potential strategy for drug-induced bone loss.

With respect to PEN, MK4 also has demonstrated an ability to significantly reduce PEN and restore bone elasticity in vivo.[126] Researchers studied the effects of MK4 on bone mechanical properties AGE crosslinking in male WBN/Kob rats, a diabetes animal model. The onset of diabetes in these rats was observed at 12 months of age with mean fasting blood glucose levels of 493 ± 119 mg/dL thereafter. Fourteen-month old male WBN/Kob rats were given either control solution or MK4 (50 mg/kg/d) for 8 or 16 weeks. Femoral 3-pont bending tests showed a significant increase in elastic modulus and toughness in rats treated with MK4, despite no changes in femoral BMD. The mechanical test results coincided with AGE crosslinking components (PYD and DPD) being increased by 141% in the MK4 group compared to control. In contrast, PEN was reduced 78% in the MK4-treated rats compared with the control group. Bone collagen and serum intact osteocalcin levels were significantly greater in the MK4 group compared to controls. These findings clearly demonstrate that MK4 decreased PEN, ameliorated the damage to collagen caused by PEN, increased collagen accumulation in bone, increased bone strength, and improved the bone turnover rate without forming AGEs.

MK4 fulfills many of the criteria required of an anabolic agent to prevent and treat BRONJ. It decreases PEN, promotes bone collagen production, and has been shown to decrease fractures caused by osteoporosis, other diseases, and numerous medications. Fostering collagen production using MK4 increases bone quality and strength, may decrease the predisposition to microfractures, and may promote the healing of microfractures when they do occur, thereby playing a fundamental role in preventing and treating BRONJ.

It's important to note that the liver uses vitamin K_1 preferentially as a clotting factor. On the other hand, other organs—such as the brain, vasculature, breasts, and kidneys—use vitamin K_2 preferentially. Coagulation studies in humans using 45 mg per day of vitamin K_2 (as MK4)[98] and even up to 135 mg per day (45 mg 3x/d) of K_2 (as MK4)[127] showed no significant increase in pathologic coagulation risk. Even doses as high as 250 mg per kg in rats did not alter the tendency for blood-clot formation to occur.[128]

The one caveat to this treatment is for people taking the blood clotting medication warfarin (Coumadin). Warfarin originally was used as a rat poison that decreased blood clots by interrupting the vitamin K–dependent clotting factors. Therefore, taking vitamin K in any amount may interfere with the actions of warfarin and increase blood clot risk.

CONCLUSION

In conclusion, the role of collagen as elaborated in the CDRH postulated in this article cannot be ignored in the pathophysiology and potential prevention and treatment of osteoporosis and BRONJ. Collagen damage is important in the pathogenesis of osteoporosis, diabetes, and cardiovascular disease and likely is the key to understanding, preventing, and treating BRONJ. Studies from the medical literature support the safety and efficacy of MK4 as a potential therapeutic agent in preventing and treating osteoporosis and BRONJ and its use should be the subject of future research.

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