VITAMIN K BEYOND COAGULATION: A ROLE IN BONE HEALTH AND DISEASE

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Abstract

Vitamin K has long been associated solely with its role in haemostasis. The last two decades brought new intriguing insights to the function of vitamin K. While the present recommendations for daily intake are based mainly on amounts required to maintain coagulation, they may not be high enough to ensure adequate functions of vitamin K not involved in blood clotting, In the present review the role of vitamin K on bone in health and disease is being discussed. Theoretical grounds connected with the function and diagnostic application of the vitamin K-dependent protein osteocalcin is described. Available clinical data related to the use of vitamin K in the prevention and treatment of osteoporosis is provided.

Key words: Vitamin K, osteocalcin, osteoporosis, supplementation

Among the exciting stories of recent developments of old drugs is the one of vitamin K (VK). Discovered by the Danish biochemist Henrik Dam in 1930 as an "antihaemorrhagic factor", it was designated as **K**oagulation (in German) vitamin ("K"). This trace nutrient was found to be present both in plant and in animal sources. Edward Doisy later distinguished the vitamin of plant origin (K1) from the one isolated from fish meal (K2). Subsequently, they shared the Nobel Prize in Physiology or Medicine in 1943.

80 years now that VK has been inevitably associated with its role in haemostasis. Part of the popularity of VK comes from the widespread clinical use of its antagonists, the coumarin anticoagulalnts.

The last two decades brought new intriguing insights to the function of VK. Research in this area has revealed a number of functions not related to coagulation, thus ascribing the quality of "omnipotent" vitamin to it [12].

Chemical structure and dietary sources

VK comprises a family of similar naphthoquinones (fig.1). The individual molecules differ from each other by the length and degree of saturation of the side chain at the 3-position of the common menadione structure.

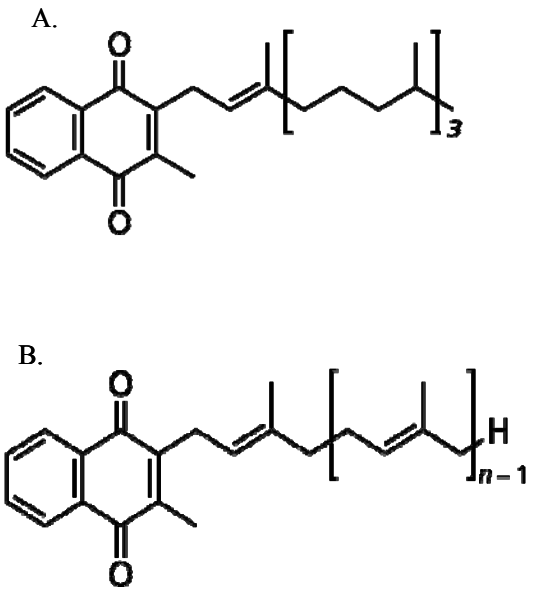

Fig. 1. Chemical structure of vitamin K: A. Phylloquinone; B. Menaquinones

In VK1, *phylloquinone*, it is a phytil side chain that is attached to the main nucleus. This form is synthesized by plants, hence the names '*phytomenadione*' or '*phytonadione*", which are used interchangeably with *phylloquinone.* The richest dietary sources are the green leafy vegetables, but it is found also in some vegetable oils, fruits, grains and dairy products.

VK2 is presented by a number of bacterially synthesized menaquinones (MKs), where the side chain comprises a polymer of repeating prenyl units. Mks are classified according to the number (n) of these units (MK-n). In humans, it has long been believed that the

main source of VK2 is the intestinal bacterial pool. Recent findings argue against the significance of intestinally derived vitamin: gut menaquinones appear to have much less importance than previously thought [31]. Dietary MKs are present in animal livers and fermented foods typically represented by cheeses in Western diets and *natto* (MK-7) in Japan. Of interest, MK-4 is considered a biosynthetic product of phylloquinone in the organism [29].

Although it is difficult to estimate the relative contribution of phylloquinone and MKs in the nutritional VK intake, studies in Netherlands suggest that VK1 constitutes approximately 90 % and MKs provide ~ 10 % of the dietary supplies [28].

Vitamin K function

Well recognized function of VK is the participation in the post-translational modification of specific proteins, known as VK-dependent proteins, e.g. clotting factors II, VII, IX and X. They share the common feature to possess gamma-glutamic residues that need to be carboxylated as a critical step for their functional activation. The reduced form of VK – hydroquinone (VKH_2) serves a co-factor of gamma-glutamyl carboxylase, which is then restored through an intermediate epoxide (VKO). The recovery is catalyzed by VKO-reductase, the target enzyme of coumarin anticoagulants. The described system is known as "VK cycle" (fig.2). The continuous recycling of VK is the reason that this nutrient is minimally stored in the organism.

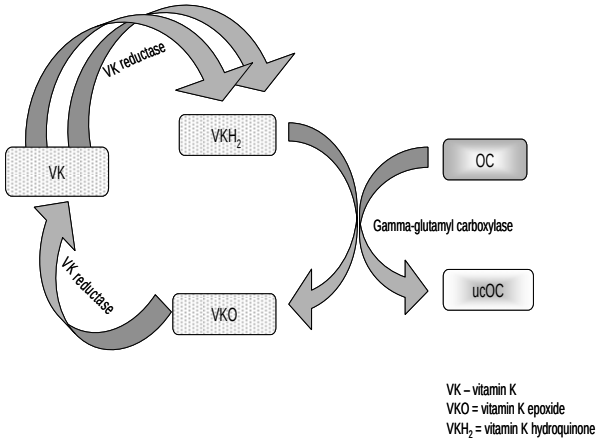

Fig. 2. Vitamin K cycle

Effects on bone

In addition to the clotting factors, various other VK-dependent proteins have been described and studied recently. Amongst those expressed in bone, osteocalcin is the most intensively investigated.

Osteocalcin is the most abundant non-collagenous bone matrix protein, secreted by mature osteoblasts. Vitamin D3 stimulates the gene transcription of OC and glucocortico-steroids suppress it [23].

OC contains 3 gamma-glutamic moieties. Although discovered more than 30 years ago, this VK- dependent protein continues to be a matter of interest and debate and its mechanism of action in bone remains obscure. Its ability to bind calcium through the negatively charged carboxylated gla residues was considered to reflect a function in bone mineralization. However, knock-out experi-ments in mice reveal that OC is not involved in bone mineralization [13]. Subsequently, using a more sensitive assay, Boskey et al. [7] did find differences in knockouts after ovariectomy compared with wild-type animals, suggesting a role of OC in mineralization maturation. Koshihara [20], on the other hand, found that in human periosteal osteoblasts in vitro, osteocalcin plays an important role in mineralization by osteoblasts. Recently, in a study aimed to elucidate the ultrastructural role of Gla proteins in bone mineralization, Amizuka [2] suggests a pivotal role of OC in the assembly of mineralized nodules.

Part of osteocalcin produced by osteoblasts and not deposited in bone matrix, escapes in the circulation [26]. Thus it can be measured in blood as a marker of osteoblasts function, i.e. of bone formation [8]. On the other hand, it has been found that OC is released in blood during bone resorption and can be viewed as a marker of increased bone turnover. Interpretations of the levels of intact OC in the evaluation of bone status are often controversial. According to Vermeer [35], for example, low levels of carboxylated osteocalcin are associated with increased risk for femoral neck fracture, while in other studies serum OC has been found to negatively correlate with BMD in postmenopausal women [21]. The undercarboxylated OC (ucOC) in serum, which is inactive in respect to bone metabolism, has

been considered a more reliable inverse marker of bone health [25]. Clinical studies have reported increased levels of ucOC in age-related change in BMD [19, 25, 32]. Particularly, ucOC has been regarded as a good predictor of hip fracture in postmenopausal women [4, 5, 30, 37]. High levels of ucOC generally reveal deficiency in VK and readily decrease upon dietary repletion or supplementation.

In addition to affecting bone metabolism through OC, menatetrenone (MK-4) and not phylloquinone, has been found to directly stimulate bone formation by a mechanism not sensitive to warfarin, suggesting that carboxylation is not the only way for VK to act on bone [3]. It has also been shown that VK improves calcium balance by stimulating intestinal calcium absorption [33] and reducing calciuria [27] in experimental rats.

Clinical studies

A number of epidemiological and prospective studies demonstrate the connection between VK and bone health. Analysis within the Nurses' Health Study cohort shows that low intake of VK may increase the risk of hip fracture in women [14]. This observation has been subsequently confirmed in smaller studies [1]. VK1 but not MK-4 and MK-7 insufficiency has been associated with increased susceptibility of vertebral fracture independently from bone mineral density [34]. VK supplementation has been shown to decrease ucOC and to reduce the risk of osteoporotic fractures, though the mechanisms are poorly understood [1, 6, 17]. It seems that VK induces only modest changes (if any) in BMD and bone turnover parameters (other than ucOC) [5], but improves bone quality and strength [2, 17]. The anti-fracture effect of VK has been shown with both MKs (predominantly MK-4 and MK-7 being used, mainly used in Japan) and phylloquinone (in Europe) [1, 11, 17]. Further, a synergism between VK and vitamin D3 with calcium, reasonable on theoretical grounds, has been demonstrated in a number of studies [1, 6, 16].

Clinical data show that nutritional deficiency of VK is common in Western world, especially in children and in elderly. The currently recommended daily intakes vary amongst countries, e.g. 80-120 mcg/day in USA or approximately 1 mcg/kg/day in the United Kingdom. Recommended intakes of VK are based solely on amounts required to maintain coagulation and they may not be high enough to ensure adequate function of VKD proteins not involved in hemostatsis. Indeed, most of the clinical studies employed relatively high doses of VK. Nutritional experts share the opinion that there is a need for establishment of a higher daily intake [9, 22, 36].

Adverse Reactions

Relatively high doses of VK have been used in the supplementation studies: 5 mg per day for phylloquinone and 45 mg for menaquinone [MK-4]. In these pharmacological doses the reported incidence of adverse effects (gastrointestinal, skin rashes) has been indistinguishable from placebo groups [1, 11]. The good long term tolerability was accompanied in one study with phylloquinone by a reduced number of cancers [11].

Oral anticoagulants and bone health.

The recognition of the VK role for bone metabolism reasonably brought the question about the effects of VK antagonists, widely used as oral anticoagulants, on bone. Numerous studies, both experimental and clinical, have addressed the issue, but so far no conclusive results have been reached [10, 18, 15, 38]. Although the literature data are ambiguous, a recent review of retrospective studies reveals that long-term therapy with anticoagulants adversely affects vertebral BMD and fracture risk. At a minimum, clinicians should carefully assess anticoagulated patients for osteoporosis risk, monitor BMD, and refer them to dietitians for dietary and supplement advice on bone health [24].

Osteoporosis is a heavy social burden to the contemporary world. Although much progress has been made in the treatment of this condition, the search for new cost-effective therapies to meet the needs of large affected populations continues. Vitamin K, alone or in combinations, may prove in the future to be a useful adjunct to the present armamentarium for osteoporosis prevention and treatment.

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