



## Complex regional pain syndrome: A vitamin K dependent entity?

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### ARTICLE INFO

#### Article history:

Received 4 March 2010

Accepted 13 March 2010

### SUMMARY

Complex regional pain syndrome (CRPS) is the complication of some injuries, such as a fracture, which affects the distal end of the injured extremity characterized by pain, allodynia, hyperalgesia, edema, abnormal vasomotor and sudomotor activity, movement disorders, joint stiffness, regional osteoporosis, and dystrophic changes in soft tissue. Exact pathogenic mechanism of CRPS is still unclear. Suggested pathogenic mechanisms of CRPS are evaluated in four major groups consist of classic inflammation, hypoxic changes and chronic ischemia, neurogenic inflammation and sympathetic dysregulation. All of these suggested pathogenic mechanisms produced by inflammatory cytokines mediated by nuclear factor kappaB. Vitamin K is a family of structurally similar, fat-soluble, 2-methyl-1,4-naphthoquinones. Vitamin K exerts a powerful influence on bone formation, especially in osteoporosis. Fat in bone stores some vitamin K. Gamma-carboxylation of the glutamic acid in osteocalcin is vitamin K dependent. Osteocalcin plays a role in calcium uptake and bone mineralization. Osteocalcin, the most abundant non-collagenous protein in bone, is produced by osteoblasts during bone matrix formation. Because osteocalcin is not carboxylated in case of vitamin K deficiency at the distal site of fracture or injury, it cannot bind to hydroxyapatite causing osteoporosis. Fracture starts a local inflammatory process in the fracture site and adjacent tissues as seen in CRPS. Vitamin K was shown to suppress the inflammatory cytokines and NF-kappaB and prevent oxidative, hypoxic, ischemic injury (which have key role in both initiation and progression of CRPS) to oligodendrocytes and neurons. We hypothesized that vitamin K has a key role and modulatory effect in CRPS pathogenesis. Vitamin K deficiency at the distal site of fracture occurs because of diminished and slowed circulation, local immobilization after extremity fracture or injury and use of vitamin K store at the distal site of the injured extremity and in the circulation for fracture healing and bone remodelling. In case of vitamin K deficiency at the distal site of fracture, classic inflammation starts with fracture at the distal tissues could not be restricted and classic inflammation, hypoxic changes, chronic ischemia, neurogenic inflammation, sympathetic dysregulation, which are the pathogenic mechanisms of CRPS, and patchy osteoporosis which occur due to high level of under-carboxylated osteocalcin could not be prevented. Briefly vitamin K level decreases in the distal site of the injured extremity consequently resulting in patchy osteoporosis due to high level of under-carboxylated osteocalcin and unrestricted inflammation which are the cause for both initiation and progression of CRPS.

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### Introduction

Vitamin K is a family of structurally similar, fat-soluble, 2-methyl-1,4-naphthoquinones, including phylloquinone (K1), menaquinones (K2), and menadione (K3). The structural difference is in the substituent R group. Phylloquinone is found in higher plants and algae, with the highest concentrations found in green leafy vegetables [1]. The most common form of vitamin K2 in humans is menaquinone-4 (MK-4), produced by intestinal bacteria from exogenous and gut bacterial naphthoquinones and transformed endogenously in our own cells [2]. Vitamins K1 and K2 differ only in the substituent R group. Menadione (K3) is not considered a nat-

ural vitamin K, but a synthetic analogue that acts as a provitamin. It possesses a much simpler structure, with no aliphatic R group chain [3]. Although menadione is considered a synthetic analogue, Billeter et al. found that phylloquinone can be cleaved by bacteria in the intestine to form a minor amount of menadione [2]. Whether colonic vitamin K can be effectively absorbed and, thus, can contribute to human vitamin K supply is still a matter of dispute [2].

Vitamin K is a cofactor in a number of biochemical pathways. The most known function of vitamin K is to support gamma (g)-carboxylation reactions of vitamin K dependent proteins. In these reactions the reduced form of vitamin K (hydroquinone) de-protonates glutamate via the gamma-glutamylcarboxylase enzyme. The epoxide formed is recycled via vitamin K epoxide reductase and quinone reductase, and glutamic acid-containing proteins, such as coagulation factors II (prothrombin), VII, IX, and X, protein C,

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and protein S, are carboxylated. Compared to the other vitamin K analogues, vitamin K2 has the most potent gamma-carboxylation activity [3,4].

Vitamin K1 is absorbed from the gastrointestinal tract in the presence of bile salts and pancreatic lipase. Once absorbed, vitamin K accumulates in the liver, spleen, and lungs, but significant amounts are not stored in the body for long periods. The action of vitamin K1, when administered parenterally, is generally detectable within 1–2 h, and hemorrhage is usually controlled within 3–8 h. A normal prothrombin level may often be obtained in 12–14 h. The pharmacokinetics of supplemental vitamin K2 is not yet clearly understood, although its clinical efficacy is evident [3]. Roles of K vitamin in bone mineralization, arterial calcification, inflammation, apoptosis, phagocytosis, cell differentiation, chemotaxis, signal transmission are now known aside from the role of which in blood clotting [5–10].

Osteoporosis is characterized by decreased bone mass and deranged microarchitecture, which contribute to bone fragility. In many previous studies, a significant correlation between reduced bone fracture and improved bone strength indices and various indices of vitamin K has been found [11,12].

These effects of vitamin K over bone are suggested to occur through vitamin K dependent protein osteocalcin. Osteocalcin, which is produced by osteoblasts during bone formation, is the primary non-collagenous protein in bone. Osteocalcin functions as a regulator of bone mineral maturation [13]. Osteocalcin is involved with calcium uptake and bone mineralization. Gamma-carboxylation of the glutamic acid in osteocalcin is vitamin K dependent and involves the conversion of glutamic acid residues (Glu) to gamma-carboxyglutamic acid residues (Gla). Osteocalcin ability to bind calcium is dependent on the vitamin K dependent gamma-carboxylation of three glutamic acid residues [14–16]. The gamma-carboxylation of osteocalcin is the primary mechanism underlying protective influence of vitamin K on bone. Vitamin K can also modulate inflammatory mediators and certain cytokines involved in bone turnover, such as osteoprotegerin and interleukin-6 [14–19].

Fat in bone stores some vitamin K and may provide this to bone cells [20]. Ultimately, however, all vitamin K is transported to bone by way of the blood circulation [21]. All natural forms of vitamin K are extremely lipophilic and therefore not miscible with aqueous solution such as blood. Vitamin K is not bound to proteins like some of the other fat-soluble vitamins but in blood is exclusively associated with lipoproteins. Vitamin K was predominantly associated with the triglyceride-rich lipoproteins (TRL); much smaller fractions were carried by low density lipoproteins (LDL) and high density lipoproteins (HDL). The TRL fraction actually comprises two families of lipoproteins: very low density lipoproteins (VLDL) and VLDL-derived lipoproteins from the liver, and chylomicrons and chylomicron-derived lipoproteins that are of intestinal origin. Upon oral consumption, radiolabeled vitamin K is absorbed from the small intestine, becomes associated with chylomicrons in blood and disappears from circulation at the same rate as chylomicrons [22]. This pattern is consistent with the assumption that chylomicrons and chylomicron remnants are the main carriers of vitamin K in blood [21,22].

The apoE genotype, which influences serum cholesterol and triglycerides, has been suggested to influence skeletal health through the transport of vitamin K to bone [21]. More specifically, it has been shown that individuals who carry the apoE4 allele and have a rapid hepatic clearance of chylomicron remnants as well as lower serum cholesterol and triglyceride concentrations, also have lower bone mineral density (BMD) and increased risk of fracture which some have attributed to inadequate vitamin K transport to the skeletal tissue [23,24].

Deficiency of vitamin K is also associated with newborn vitamin K deficiency bleeding, obstructive icterus, pancreas and hepatobiliary diseases and malabsorption syndromes [25–27].

Especially, fat malabsorption in which bile acids have an important role leads to vitamin K deficiency. Bile acids aid absorption of fat, consequently aid absorption of fat-soluble vitamin K. Therefore, defects in the synthesis and/or release of bile acids may result in vitamin K deficiency [26]. Furthermore, some drugs may cause vitamin K deficiency, for example anticoagulants (heparin, warfarin, dicoumarol) via antagonizing vitamin K, antibiotics via eliminating intestinal bacteria which produce vitamin K [26,28]. Particularly, events such as upper extremity trauma or stroke that effect nutrition negatively cause insufficient vitamin K uptake in diet and concurrently using drugs that make vitamin K deficiency may introduce the risk of vitamin K deficiency.

Complex regional pain syndrome (CRPS) is complication of injuries, such as a fracture or sprain, which affects the distal end of the injured extremity characterized by pain, allodynia, hyperalgesia, edema, abnormal vasomotor and sudomotor activity, movement disorders, joint stiffness, regional patchy osteoporosis, and dystrophic changes in soft tissue [29]. There are two subtypes of CRPS based on the absence (type 1) or presence (type 2) of direct nerve injury [30].

Exact pathogenic mechanism of CRPS is still unclear. Suggested pathogenic mechanisms in CRPS are evaluated in four major groups [31]. Classic inflammation, the first pathogenic mechanism, in CRPS is a local increase of the cytokines TNF-alpha, IL-6, and tryptase [32–34].

These cytokines can activate or are activated themselves by transcription factor nuclear factor kappaB (NFkB) [35]. Hypoxic changes and chronic ischemia, the second pathogenic mechanism in CRPS induce NFkB activation, mediated by the formation of reactive oxygen species and peroxynitrite [36]. Neurogenic inflammation, the third pathogenic mechanism in CRPS, is initiated by neuropeptides including substance P (SP), Calcitonin Gene Related Protein (CGRP), Vasoactive Intestinal Protein (VIP), Bradykinin, Neuropeptide Y [37,38].

NFkB interacts with neuropeptides such as CGRP and SP [31]. Several animal models demonstrate the role of NFkB in pain induction and pain maintenance in the central nervous system (CNS). Additionally, NFkB in the CNS mediates IL-1 induced COX-2 up-regulation and prodynorphin expression [31]. The fourth pathogenic mechanism in CRPS, sympathetic dysregulation induced by catecholamines mediated by NFkB [31]. Hettne et al. have revealed, after automated computer analysis of the literature, that the transcription factor nuclear factor kappaB (NFkB) is involved in all these four disease mechanisms and is a central mediator in both initiation and progression of CRPS [31]. In a recent study using a chronic postischemia pain (CPIP) rat model of human CRPS type 1, it has been demonstrated that increased NFkB activity in muscle and spinal cord [39].

It has been reported that high vitamin K status was associated with lower concentrations of inflammatory markers [19]. And Ide et al. showed that vitamin K2 inhibits MMP expression by suppressing NF-kappaB and MAP kinase activity [40]. These observations suggest that a possible protective role for vitamin K in inflammation seen in suggested CRPS pathomechanisms.

## The hypothesis

We hypothesized that vitamin K has a key role in CRPS pathogenesis. Vitamin K store at the distal site of the injured extremity would be reduced because of diminished, slowed circulation, local immobilization after extremity fracture or injury. Use of vitamin K stores and vitamin K in the circulation for

fracture healing and bone remodelling at the site of fracture, and probably also migration of vitamin K from the distal site of fracture to the site of fracture results in vitamin K deficiency consequently high level of under-carboxylated osteocalcin levels at the distal site of fracture. In vitamin K deficiency, classical inflammation starts with fracture in the distal adjacent tissue could not be restricted and hypoxic changes, chronic ischemia, neurogenic inflammation and sympathetic dysregulation which play roles in the pathogenic mechanisms of CRPS could not be prevented in the state of vitamin K deficiency at the distal site of fracture. Consequently focal patchy osteoporosis seen in CRPS occurs because of high level of under-carboxylated osteocalcin and inflammation due to vitamin K deficiency at this site. Briefly, vitamin K level decreases in the distal site of the injured extremity consequently resulting in patchy osteoporosis due to high level of under-carboxylated osteocalcin and unrestricted inflammation which are the cause for both initiation and progression of CRPS.

### Evaluation of the hypothesis

Following points can be considered to support this hypothesis.

#### *Vitamin K suppresses inflammatory markers and NF-kappaB*

NFkB and other inflammatory mediators, which are required for both initiation and progression of CRPS, could not be suppressed in the lack of vitamin K causing CRPS at the distal site of the injured extremity. Vitamin K may play a modulatory role in fracture healing. Fracture starts a local inflammatory process in the fracture site and adjacent tissues as seen in CRPS. Interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) are known to play a role in initiating the repair cascade of bone fracture [41,42]. It has been observed that high vitamin K status was associated with the low concentrations of inflammatory markers suggesting a possible protective role for vitamin K in the inflammation occurs in CRPS pathomechanisms [19,31]. Hettne et al. have revealed, after automated computer analysis of the literature, that the transcription factor nuclear factor kappaB (NFkB) is involved in all suggested inflammatory CRPS disease mechanisms and is suggested a central mediator in both initiation and progression of CRPS [31]. Ide et al. showed that vitamin K inhibits MMP expression by suppressing NF-kappaB and MAP kinase activity [40]. And Tanaka et al. found that vitamin K attenuates lipopolysaccharide-induced acute lung injury through inhibition of nuclear factor kappaB activation [43].

#### *Vitamin K prevents hypoxic changes, chronic ischemia, neurogenic inflammation, sympathetic dysregulation*

Hypoxic changes, chronic ischemia, neurogenic inflammation and sympathetic dysregulation are the known pathogenic mechanisms of CRPS as mentioned above. Vitamin K may suppress these pathogenic mechanisms. Because Li et al. showed that vitamin K prevents oxidative, hypoxic, ischemic injury to oligodendrocytes and neurons and also showed prevents hypoxic, ischemic cell death by inhibiting activation of 12-lipoxygenase in oligodendrocytes [44,45]. And Isaev et al. showed vitamin K reduces rotenone-induced cell death in cerebellar granule neurons via decrease of rotenone-induced free radical production and antioxidant [46]. Hypoxic changes, chronic ischemia, neurogenic inflammation and sympathetic dysregulation which are the pathogenic mechanisms of CRPS could not be prevented in the state of vitamin K deficiency at the distal site of fracture.

#### *Vitamin K is used up for clotting to cease bleeding after the trauma*

Clotting proteins (factors II, VII, IX, X) of which vitamin K is essential cofactor are used up to cease of bleeding in hemorrhagic traumas or operations. Unlike blood coagulation proteins, which need much lower levels of vitamin K for complete gamma-carboxylation, higher levels of vitamin K are essential for the total gamma-carboxylation of osteocalcin [47]. Since insufficient level of vitamin K at the distal site of fracture or injury results in high level of under-carboxylated osteocalcin and consequently focal osteoporosis which occurs in CRPS.

#### *Vitamin K is used up for the fracture healing and bone remodelling at the site of fracture*

Vitamin K has been shown to be the most important inducer of bone mineralization in human osteoblasts [48]. In vitro studies using assays from various species demonstrate also vitamin K inhibits osteoclastogenesis of bone [49]. Vitamin K has also a transcriptional regulatory function. The up-regulation in the expression of bone markers in vivo suggests an anabolic action of vitamin K [50]. Osteocalcin, the most abundant non-collagenous protein in bone, is produced by osteoblasts during bone matrix formation. Because osteocalcin is not carboxylated at the distal site of fracture or injury in case of vitamin K deficiency, it cannot bind to hydroxyapatite, causing patchy osteoporosis seen in CRPS. Briefly, vitamin K is used up for the bone remodelling at the site of fracture. Vitamin K at the distal site of fracture may migrate to the site of fracture. Vitamin K level decreases in the distal site of the injured extremity resulting in patchy osteoporosis and CRPS.

#### *Antibiotics may eliminate intestinal vitamin K source*

Antibiotics used for the prevention of infection at the site of injury may eliminate intestinal bacteria which are the endogenous vitamin K source [50]. In this case, if intake of vitamin K with diet is insufficient, vitamin K deficiency may occur at the distal site of fracture or injury.

#### *Anticoagulant drugs antagonise the effects of vitamin K*

Antagonized vitamin K action at the distal site of fracture or injury may result in CRPS. Vitamin K antagonists such as warfarin also increase the risk of osteoporosis [28]. Anticoagulants (heparin, warfarin, dicoumarol, etc.) used to reduce the risk of thromboembolic as a result of posttraumatic or postoperative immobilization antagonises the effects of vitamin K on the bone.

#### *Vitamin C and bile acids provide the absorption of vitamin K from intestine*

Vitamin C supplementation after trauma has been showed to prevent CRPS in some studies [51,52]. The mechanism of this effect was suggested by the authors to be its antioxidative action. However, antioxidative effect of vitamin C alone does not explain its preventive action in CRPS. We suggest that this effect of vitamin C occurs via increased absorption of vitamin K. Vitamin C is essential for the bile acid synthesis at the level of 7 alpha hydroxylation [53]. Bile acids are required for the absorption of fatty acids and vitamin K [3,53]. Therefore, preventive effect of vitamin C in CRPS may occur via the increased absorption of vitamin K.

#### *Local immobilization and diminished and slowed circulation*

Immobilization is also a risk factor of disuse osteoporosis. Victims of stroke are often immobilized, leading to significant loss

of BMD. The most pronounced loss of bone occurs on the hemiplegic side compared to the un-affected side. This loss has been attributed to increased bone resorption, immobilization-induced hypercalcemia, and hypovitaminosis D [54]. A recent randomized, controlled trial of 108 hemiplegic stroke victims, 54 subjects treated with 45 mg vitamin K2 daily for 12 months and 54 subjects serving as controls, found K2 effective for preventing disuse bone loss. Second metacarpal BMD on the hemiplegic side increased an average of 4.3% with vitamin K2 and decreased an average of 4.7% with no treatment. The un-affected side had a 0.9% decrease with K2 treatment compared to 2.7% decrease with no treatment [55]. And Iwasaki-Ishizuka et al. found that vitamin K reserves bone loss by improving osteoblast dysfunction in rats immobilized by sciatic neurectomy [56]. Vitamin K can be seen in blood at approximately 4 h after intake, and half life of which is approximately 72–96 h [57]. Because of diminished–slowed circulation and local immobilization, vitamin K store at the distal site of the injured extremity would be run out due to its 72–96 h half life resulting in vitamin K deficiency consequently inadequate carboxylated osteocalcin at the distal site of fracture leading to CRPS.

#### *Insufficient vitamin K uptake in diet*

Particularly, conditions such as upper extremity trauma or stroke that affect nutrition negatively and concurrently using drugs that lead to vitamin K deficiency may produce the risk of vitamin K deficiency and consequently leading to CRPS.

#### **Testing the hypothesis**

We suggest the following approaches to test some aspects of the hypothesis:

1. Set an animal model of CRPS type 1 (for example chronic post ischemic pain rat model (CPIP model)).
2. Measure vitamin K, under-carboxylated osteocalcin, some of inflammatory cytokines and NFκB levels in vitamin K treated and non-treated groups in nuclear extracts of affected tissues, un-affected tissues and spinal cord tissue using ELISA. Evaluate CRPS clinical findings in vitamin K treated and non-treated groups.
3. Make the statistical analysis of the results.

#### **Conclusion**

Vitamin K plays an important role in the CRPS pathogenesis according to our hypothesis. Vitamin K deficiency at the distal site of the injured extremity seems to be responsible factor for CRPS. Points mentioned above each alone may not constituted the vitamin K deficiency at the distal site of the injured extremity. But, after an extremity trauma or injury, all or some of these points may together play a role in vitamin K deficiency at the distal site of injured extremity. Considering the explained pathogenic mechanisms of CRPS and role of vitamin K deficiency in these pathogenic mechanisms, it can be said that CRPS occurs at the distal site of injured extremity.

#### **Conflicts of interest statement**

None declared.

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