

Vitamin K2 supplementation in haemodialysis patients

Abstract

Cardiovascular disease is the leading cause of morbidity and mortality in patients with end-stage renal disease undergoing haemodialysis. In these patients there is a high incidence of severe arterial calcification, and this in turn has been linked to vitamin K2 deficiency. In this article we have therefore reviewed the potential role of vitamin K2 supplementation in reducing arterial calcification, and hence the risk of cardiovascular mortality, in haemodialysis patients.

Key words: haemodialysis; vitamin K2; cardiovascular disease; arterial calcification.

Introduction

Cardiovascular disease is the leading cause of morbidity and mortality in patients with end-stage renal disease undergoing haemodialysis (Quack & Westenfeld, 2016). In these patients there is a high incidence of severe arterial calcification, and this in turn has been linked to vitamin K2 deficiency (Blacher et al, 2001; Jean et al, 2009; Noordzij et al, 2011). In this article we have therefore reviewed the potential role of vitamin K2 supplementation in reducing arterial calcification, and hence the risk of cardiovascular mortality, in haemodialysis patients.

Vitamin K basics

The term vitamin K refers to a group of fat-soluble substances of related chemical structure. Vitamins K1 and K2 are of natural origin; other types of vitamin K (e.g. vitamin K3, menadione) are synthetic. In terms of evidence from controlled clinical trials, vitamin K of natural origin is considered to be

superior (in terms of efficacy and safety) to the synthetic forms. Concerns have been raised regarding possible toxicity of menadione, the synthetic form vitamin K3 (Chiou et al, 1997). Vitamin K1 (also known as phylloquinone) is mainly of plant origin, vitamin K2 (also known as menaquinone) is mainly of animal origin, being produced by bacteria in the intestinal tract (either in farm animals or man). In man, approx 50% of the body's K2 requirement is obtained from the diet, and 50% synthesised within the human digestive tract (Schurgers et al., 2000). A form of vitamin K2 obtained from the Japanese fermented soybean food natto (a uniquely rich dietary source of K2) is called Menaquinone-7 (MK-7), the importance of which will be discussed in more detail in this paper. An adequate intake of vitamin K1 can be achieved by regular consumption of green leafy vegetables such as spinach and kale. An adequate intake of vitamin K2 is more difficult to achieve; vitamin K2 can be obtained from eggs and dairy products, with estimated values of 70mcg per 100g cheese, 30mcg per 100g egg yolk and 15mcg per 100g butter. It is important that these foods are obtained from grass-fed cows or hens, since produce from intensively reared livestock contain substantially lower levels of vitamin K2. The values for the above foods compare with a vitamin K2 content of approximately 1100mcg per 100g of natto.

Functions of vitamin K2

Both vitamin K1 and K2 are converted within the body into the active form dihydrovitamin K, which in turn is a key co-factor of the enzyme gamma glutamyl carboxylase. Gamma glutamyl carboxylase activates (via carboxylation) calcium binding proteins involved in blood clotting, bone

structure and cardiovascular function. Of the two natural forms of vitamin K, vitamin K2 is considered to be superior to vitamin K1, in terms of bioavailability and effectiveness within the body (Schwalfenberg, 2017). For example, vitamin K2 (in MK-7 form) is more easily absorbed from the digestive tract, is retained longer within the body, has a wider tissue distribution, and has greater metabolic action, compared to vitamin K1 (Schurgers et al, 2007).

Intestinal absorption of vitamin K2 occurs as for other lipid soluble substances. In the duodenum, vitamin K2 is subject to the process of micellisation, through which lipid soluble substances are prepared for intestinal absorption. Bile from the gall bladder is secreted into the duodenum, and substances present in bile interact with vitamin K2 molecules to form characteristic micellular spherical structures up to 20nm in diameter. Micelles are small enough to diffuse between intestinal villi, thereby transporting vitamin K2 to the surface of enterocytes prior to absorption. Enterocyte cells forming the lining of the small intestine villi absorb vitamin K2 via a process of passive diffusion (passive means the process does not require energy). This passive diffusion process is influenced by levels of unsaturated fatty acids, bile salts and luminal pH, factors relevant to patients who may have undergone bowel surgery. Once inside the enterocyte, vitamin K2 is incorporated into chylomicrons, lipoprotein based particles designed for the transport of lipid soluble substances in the circulation. Chylomicrons are released by exocytosis from the basal surface of enterocytes into the lymphatic system. Vitamin K2 containing chylomicrons eventually access the subclavian vein (via the proximal abdominal and thoracic lymph ducts), for systemic circulation.

following first pass repackaging in the liver via binding to LDL cholesterol for systemic transport within the bloodstream. On this basis, vitamin K2 supplements are most efficiently absorbed in capsule form with a carrier oil matrix, which promotes micelle formation (Knapen et al, 2014).

Patients with bone weakening often had calcified deposits within their blood vessels was recognised more than 100 years ago, although the role of vitamin K2 in this process was not understood at the time. Vitamin K2 activates the proteins matrix Gla protein (MGP) and osteocalcin, which transfer calcium from the lining of arteries into the bone matrix respectively (Villa et al, 2017). In addition to bone disorders, deficiency of vitamin K2 results in the deposition of calcium in soft tissues such as the heart, and in blood vessel walls, and is therefore associated with an increased risk of cardiovascular disorders- this results from weakening of heart muscle and/or valves, stiffening of artery walls, plaque formation and atherosclerosis. Vitamin K2 has also been shown to have an important role in normal immune function. Vitamin K2 inhibits the proliferation of cancer cells, and deficiency has been associated with an increased risk of prostate, liver and lung cancers (Kakizaki et al, 2007; Hey & Brasen, 2015).

Vitamin K2 deficiency

A vitamin K deficiency can result from poor diet, alcoholism, smoking, malabsorption syndromes and the use of certain types of antibiotic. In particular, vitamin K2 levels may be depleted by the use of statins (Okuyama et al, 2015). Nutritional experts consider that a majority of the UK population

are vitamin K2 deficient to some degree- levels of vitamin K considered sufficient to maintain normal blood clotting may not be sufficient to maintain healthy bone structure and cardiovascular function. Vitamin K2 levels are essentially nil in processed convenience foods, and low even in a healthy diet. As noted above, depletion of vitamin K2 is associated with osteoporosis, cardiovascular disease, abnormal blood clotting and increased risk of some types of cancer.

A number of clinical studies have reported a link between vitamin K2 deficiency, arterial calcification and risk of morbidity/mortality. A study of the dietary intake of vitamins K1 and K2 in some 5000 subjects over a ten year period found that those in the highest third of vitamin K2 intake (the vitamin K2 intake ranged from < 21.6 mcg/day in the lowest third to > 32.7 mcg/day in the highest third of menaquinone (Vitamin K2) intake) were 52% less likely to develop severe calcification of the arteries, 41% less likely to develop cardiovascular disease, and 57% less likely to die from cardiovascular disease. (Geleijnse et al., 2004). However, intake of vitamin K1 had no effect on these cardiovascular disease outcomes. Similarly, a study of vitamin K1/K2 intake in 16,000 women aged 50-70 years found that participants with the highest intake of vitamin K2 had a much lower risk of cardiovascular disease; for every 10 micrograms of K2/day consumed, the risk of cardiovascular disease was reduced by 9% (Gast et al, 2009).

In haemodialysis patients, vitamin K deficiency may result from poor dietary intake, in part due to the dietary limitations imposed by the uremic status (Cranenburg et al, 2012; Fusaro et al, 2017), smoking (Fusaro et al, 2017),

use of statins (Chen et al, 2017), time on dialysis (Feng et al, 2015), or treatment with vitamin K antagonists (Caluwe et al, 2016; Voskamp et al, 2018). Use of vitamin K antagonists (coumarins; principally warfarin, but also phenindione and acenocoumarol) in haemodialysis patients has been linked to various adverse outcomes (De Mauri et al, 2017). Vitamin K deficiency has been linked to the development of calciphylaxis, an uncommon but frequently fatal complication in haemodialysis patients (Nigwekar et al 2017). In this small case-control study comprising 40 haemodialysis patients, calciphylaxis was associated with a significant reduction in vitamin K mediated carboxylated matrix Gla protein, which is required to inhibit vascular calcification. A randomised controlled clinical study is currently in progress to determine whether supplementation with vitamin K can increase carboxylated Gla protein levels in calciphylaxis patients (NCT 02278692).

Unlike the situation with vitamin D3, there is no simple blood test to determine whether an individual may be deficient in vitamin K2. A specialised assay is available which quantifies the level of uncarboxylated matrix Gla protein, which correlates with the level of vitamin K. The European Food Safety Authority (EFSA) have not established an NRV (Nutrient Reference Value) for vitamin K2, although an adequate intake value has been listed (EFSA Panel on Dietetic Products Nutrition and Allergies (NDA), 2017). However, the adequate intake value is thought to relate to the liver requirement only (ie for adequate blood clotting). Theuwissen et al (2014) provided evidence for a deficiency of vitamin K2 in the general population, on the basis that both children and adults had high blood levels of under-carboxylated extra-hepatic proteins MGP and

osteocalcin (ie those involved in cardiovascular /bone calcium mobilisation).

Vitamin K2 supplementation in haemodialysis patients

A number of studies in haemodialysis patients have demonstrated deficiency of vitamin K2 and increased levels of uncarboxylated inactive Gla protein and/or undercarboxylated osteocalcin (Pilkey et al, 2007; Holden et al, 2011); Westenfeld et al, 2012; Bentkowski et al, 2013). The levels of uncarboxylated matrix Gla protein correlated with vascular calcification and arterial stiffness in end-stage renal disease patients (Thamrattnopkoon et al, 2017).

A clinical study of 200 haemodialysis patients by Caluwe et al (2014) demonstrated that supplementation with vitamin K2 for 8 weeks significantly (P = 0.023) decreased the circulatory level of the inactive (uncarboxylated) form of vitamin K dependent MGP in a linear and dose dependent fashion, thereby helping to prevent vascular calcification. This study is however limited by the absence of a placebo group, although, in nine patients who withdrew from the study within the first week, the plasma level of inactive MGP remained unchanged. A randomised controlled trial by Caluwe et al (NCT02610933) is currently underway to determine whether the use of the anticoagulant rivaroxaban (instead of warfarin) can slow vascular calcification, and also whether addition of vitamin K2 to rivaroxaban can further slow or prevent vascular calcification. The greatest benefit being obtained at a dose equivalent to 360 mcg MK-7/day. Similarly Westenfeld et al (2012) and Aoun et al (2017) reported supplementation with 360mcg/day vitamin K2 MK7 for 4-6 weeks in haemodialysis patients reduced mean uncarboxylated matrix Gla

protein by 86% and 93% respectively. In a study of haemodialysis patients in which vitamin K was incorporated into the dialysis buffer, over a period of 3 months arterial calcification was significantly reduced (Li et al, 2017).

Two randomised controlled trials, VitaVasK (Krueger et al, 2014) and iPACK HD (Holden et al, 2015), are in progress to determine the effect of vitamin K supplementation on the progression of arterial calcification in haemodialysis patients; however both trials are using supplemental vitamin K in K1 form, rather than K2 form.

It is of note that in patients with chronic kidney disease (stages 2-5) but not yet requiring haemodialysis, plasma levels of uncarboxylated Gla (gamma-carboxyglutamic acid-containing) protein increased progressively with the disease stage (Schurgers et al, 2010).

Osteoporosis is another common comorbidity in haemodialysis patients, with a four-fold increased risk of hip fracture compared with the general population (Iwasaki et al, 2017). While there is increasing evidence for the benefits of vitamin K2 supplementation on bone health in the general population (Huang et al, 2015), clinical trials on vitamin K2 for osteoporosis management among haemodialysis patients are generally lacking. However, two clinical studies to date have shown high dose (45mg/day) vitamin K2 to have a positive impact on markers of bone health in CKD patients. In the first study (Yonemura et al, 2000; Yonemura et al, 2004), oral supplementation with vitamin K2, alone or in combination with vitamin D3, significantly reduced prednisolone induced

bone mineral loss in patients with chronic glomerulonephritis. In the second study (Sugimoto et al, 2002), oral supplementation with vitamin K2 reduced bone mineral loss in ambulatory peritoneal dialysis patients. It should be noted that the latter study comprised only 8 patients, and that both the above studies used a form of vitamin K2 known as menatetrenone (MK-4), a less bioavailable form of vitamin K2 compared to the MK-7 form (Schurgers et al, 2007).

Safety of Vitamin K2 supplementation

In terms of safety, clinical studies have shown supplementation with vitamin K2 to be well tolerated, with incidence of adverse effects similar to placebo. With regards to long-term safety, randomised controlled clinical trials typically supplement vitamin K2 for 1-5 years (e.g. Vossen et al, 2015), although beneficial effects may be seen after 2-3 months (Caluwe et al, 2014). The safety of supplemental vitamin K2 for the general population has been confirmed in a recent pharmacopeial review (Marles et al, 2017). Vitamin k2 may interfere with warfarin based anticoagulation therapy, although supplementation of some vitamin K at a steady level during anticoagulation therapy may result in a more stable INR that requires fewer adjustments (Lurie et al., 2010).

Newer anticoagulation agents such as dabigatran, rivaroxaban, and apixaban are not vitamin K-dependent.

It is important that any oral supplement used is manufactured to pharmaceutical standards (for which certificates of analysis should be

available). In the UK food grade supplements are not subject to the same regulatory requirements as pharmaceutical medicines, and may contain substantially less vitamin K2 than stated on the product labelling. In 2013 Kappa Bioscience (a manufacturer of supplemental vitamin K2) undertook an analytical program by testing 100 vitamin K2 products for label claim. These products were purchased from retailers in a variety of product formats and formulations. Of these products, 81% did not meet the stated label claim for vitamin K2 MK-7 content, with ten products having no detectable vitamin K2 content. This is because as a lipid-soluble vitamin, vitamin K2 is susceptible to degradation, particularly when in combination with minerals such as calcium or magnesium (see www.kappabio.com/k2 stability). In addition to the above, supplements should contain 100% vitamin K2 MK-7 in the trans -form, since this is the biologically active isomer (the cis- form is biologically inactive).

Summary

Vitamin K2 supplementation is a safe and cost-effective nutritional strategy that may be integrated into the care of haemodialysis patients; vitamin K2 supplementation has been shown to reduce uncarboxylated matrix Gla protein in haemodialysis patients, and may help reduce vascular calcification. There is currently no consensus as to the dosage of vitamin K2 to be supplemented in haemodialysis patients; however guidance can be obtained from the clinical studies described in this review which typically supplemented 360mcg vitamin K2/day. On those days on which patients are dialysed, the supplement is administered immediately after the dialysis session (Caluwe et al, 2013; Aoun

et al, 2017).

Key points

1. Cardiovascular disease is a major cause of morbidity and mortality in patients undergoing haemodialysis.
2. Cardiovascular disease in haemodialysis patients has been linked with severe arterial calcification.
3. Severe arterial calcification in haemodialysis patients, and in other disorders, has been linked with a deficiency of vitamin K2.
4. Vitamin K2 is a cofactor of the enzyme gamma glutamyl carboxylase, which has a key role in activating proteins responsible for transferring calcium from arterial linings into bone.
5. Oral supplementation with vitamin K2 has the potential to reduce arterial calcification and hence risk of cardiovascular mortality in haemodialysis patients.

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