ABSTRACT

Background. Patients on haemodialysis (HD) exhibit increased cardiovascular mortality associated with accelerated vascular calcification (VC). VC is influenced by inhibitors such as matrix Gla protein (MGP), a protein activated in the presence of vitamin K. HD patients exhibit marked vitamin K deficiency, and supplementation with vitamin K reduces inactive MGP levels in these patients. The VitaVasK trial analyses whether vitamin K1 supplementation affects the progression of coronary and aortic calcification in HD patients.

Methods. VitaVasK is a prospective, randomized, parallel group, multicentre trial (EudraCT No.: 2010-021264-14) that will include 348 HD patients in an open-label, two-arm design. After baseline multi-slice computed tomography (MSCT) of the heart and thoracic aorta, patients with a coronary calcification volume score of at least 100 will be randomized to continue on standard care or to receive additional supplementation with 5 mg vitamin K1 orally thrice weekly. Treatment duration will be 18 months, and MSCT scans will be repeated after 12 and 18 months. Primary end points are the progression of thoracic aortic and coronary artery calcification (calculated as absolute changes in the volume scores at the 18-month MSCT versus the baseline MSCT). Secondary end points comprise changes in Agatston score, mitral and aortic valve calcification as well as major adverse cardiovascular events (MACE) and all-cause mortality. VitaVasK also aims to record MACE and all-cause mortality in the follow-up period at 3 and 5 years after treatment initiation. This trial may lead to the identification of an inexpensive and safe treatment or prophylaxis of VC in HD patients.

Keywords: haemodialysis, matrix Gla protein, vascular calcification, vitamin K

INTRODUCTION AND RATIONALE

Patients on haemodialysis (HD) suffer from extensive cardiovascular calcifications (VCs). VC is an independent risk factor and might explain the excessively increased cardiovascular mortality in this population [1, 2]. In the past, the development of VC was discovered to be actively regulated and influenced by inhibitors of calcification [e.g. matrix Gla protein...
(MGP), fetuin-A) [3-5]. MGP is a powerful vascular wall-based inhibitor of VC. It is produced by vascular smooth muscle cells and requires vitamin K-dependent post-translational modification, namely gamma carboxylation, to be fully active. The role of MGP was discovered in knock-out mice, that died from rupture of a massively calcified aorta [6]. Warfarin, a vitamin K antagonist, inhibits the activation of MGP thereby mimicking the MGP knock-out phenotype. Indeed, functional vitamin K deficiency, induced by warfarin, accelerates VC in the normal population [7] and in HD patients [8], as well as in rodents [9]. Warfarin is also a potent risk factor for the development of calciphylaxis, a life-threatening complication in HD patients characterized by calcified cutaneous vessels [10].

In rodents, warfarin-induced VC can be inhibited and even regressed by subsequent administration of vitamin K2 [9, 11]. In a trial in elderly non-chronic kidney disease (CKD) patients, daily administration of 500 µg vitamin K1 (phyloquinone) failed to affect the progress of coronary artery VC on an intention-to-treat basis. In subgroups with >85% compliance one) failed to affect the progress of coronary artery VC on an intention-to-treat basis. In subgroups with >85% compliance and in those with some degree of baseline VC, however, the progress of calcification was significantly retarded [12]. The administration of vitamin K1 in this trial also reduced elevated levels of undercarboxylated (uc)MGP and undercarboxylated osteocalcin (OC). In another trial, supplementing 500 µg/day vitamin K1 for 3 years in 190 individuals reduced circulating levels of ucMGP from 485 to 97 pmol/L but failed to reduce or slow progression of coronary calcification. A possible explanation is that this trial was conducted in non-CKD patients with little baseline VC [13], and thus, its design may not have been suitable to detect small changes.

At present, there is no clinical evidence supporting the hypothesis that vitamin K supplementation attenuates the progression of VC in HD patients. These patients exhibit reduced vitamin K intake [14], and uraemia, at least experimentally, also interferes with vitamin K recycling (similar to warfarin; Kaezler et al. under submission). Indeed, the levels of another vitamin K-dependent protein, PIVKA-II [a protein induced by vitamin K absence or antagonism factor II (prothrombin)], which are normally below the detection limit in healthy serum, are elevated in 97% of all HD patients [15]. In combination with the elevated uncarboxylated MGP and OC, HD patients, therefore, represent a population with a very high overall vitamin K deficiency. Taken together with their massively increased VC prevalence, they represent an ideal population for proof-of-concept trials involving the vitamin K system. Recently, we demonstrated that supplementation of vitamin K in HD patients induces a rapid decrease (on average 60%) of ucMGP, but also ucOC and PIVKA-II in serum over a 6-week period [16]. We observed almost no evidence for biochemical non-responders, indicating that all patients suffered from functional vitamin K deficiency and thus, incomplete MGP carboxylation.

Vitamin K1 is a registered and approved drug worldwide (e.g. KA-Vit®, Infectopharm, Heppenheim, Germany). Among others, it is approved for newborns and infants, as well as during pregnancy and in breast-feeding women [17]. No known toxicity exists for vitamin K1 in adults. Side effects are very rare and have only been reported after intravenous or intramuscular administration: anaphylactic reactions, venous irritation, sclerodermiform skin infiltration and pigmentation. Particularly, no increased risk of thromboembolism occurred during trials administering vitamin K [18].

In VitaVasK, we administer vitamin K1 orally. The daily recommended allowance of phylloquinone in the European Union is 75 µg. Thus, our weekly dose of 15 mg exceeds the weekly recommended vitamin K1 allowance 29-fold. However, there are no safety concerns with such doses since, apart from the lack of toxicity of even very high doses [19], other clinical studies have administered similar vitamin K1 amounts (0.5–1 mg/day) for up to 36 months without observing treatment-emergent adverse effects [20–22]. Finally, given that HD patients exhibit significantly lower vitamin K levels than non-CKD individuals [16, 23, 24], we feel that a high dosage may be justified in this population, and that the possible advantages of high-dose oral vitamin K1 replacement outweigh the potential risks in HD patients.

**TRIAL DESIGN**

The VitaVasK study is a randomized, prospective, multicentre, and open-label interventional clinical trial. The study protocol has been submitted to the relevant local ethics committees for final approval. The study is in adherence with the Declaration of Helsinki. Two treatments will be compared in different groups providing a two-arm parallel group design with 348 patients in total. The study design is illustrated in Figure 1. Due to the very specific taste and yellowish appearance of the oily liquid vitamin K1 (phyloquinone), an adequate placebo would be challenging; therefore, the study will be performed open-labelled. However, because the primary end points—i.e. thoracic aortic and coronary calcification—will be objectively measured, and because the radiologists evaluating the computed tomography (CT) scans will be blinded as to the treatment, information bias is excluded.

**TRIAL OBJECTIVES AND PURPOSE**

The first major question is whether oral supplementation of vitamin K1 is able to slow the progression of VC in the coronary and thoracic aorta in HD patients. Further questions are whether this treatment is also able to slow aortic and mitral valve calcification and regress the extent of coronary and thoracic aortic VC as well as reducing the major adverse cardiovascular events (MACE) and all-cause mortality.

The total expected trial duration is 2.5 years. The recruitment of patients is scheduled to start in October 2013 and should take 1 year. All patients who are eligible and who have signed the informed consent will be screened by a first multi-slice computed tomography (MSCT) scan of the heart and thoracic aorta. Patients who are eligible after this scan will be objectively assigned the informed consent will be blinded as to the treatment, information bias is excluded.
**INCLUSION CRITERIA**

Patients with the following criteria will be included in the trial:

(i) Males or females ≥ 18 years of age,
(ii) not less than 6 months on HD,
(iii) presence of significant coronary calcification (coronary artery volume score of >100) in the baseline MSCT,
(iv) signed informed consent,
(v) life expectancy not less than 18 months.

**EXCLUSION CRITERIA**

Patients who meet any one of the following exclusion criteria will not be included in the trial:

(i) History of thrombosis,
(ii) intake of vitamin K antagonists (coumarins, e.g. Mar-cumar®) at baseline or in the 3 months prior to baseline,
(iii) inflammatory bowel disease,
(iv) short-bowel syndrome,
(v) liver dysfunction,
(vi) haemoglobin <70 g/L,
(vii) alcohol or drug abuse,
(viii) presence of coronary stent,
(ix) women who are pregnant or breast feeding,
(x) people who are accommodated in an institution by court or administrative order,
(xi) mental condition rendering the person unable to understand the nature, scope and possible consequences of the study,
(xii) people unlikely to comply with the protocol, e.g. uncooperative attitude, inability to return for follow-up visits and unwillingness of completing the study,
(xiii) any other illness or medical treatment that could interfere with the assessment of safety, tolerability and efficacy,
(xiv) simultaneous participation in another trial or interfering examination or participation in a study within 90 days or five half-lives of an appropriate study drug (whichever is longer) prior to screening,
(xv) lack of safe contraceptive measures.

**TREATMENT DETAILS**

All patients will undergo a first MSCT scan of the chest including the coronaries and thoracic aorta. Patients with a minimum coronary calcification score (volume score; see below) of 100 will enter the trial and be randomized into one of the two arms. The threshold of 100 was chosen because larger treatment effects are seen in patients with pronounced calcification than in those with low calcification scores [25]. Using an even higher calcium score at baseline, i.e. > 100, is supposed to hamper patient recruitment. Patients in both arms will continue their standard treatment care. Patients in arm two will additionally receive vitamin K1 (phylloquinone) at 5 mg orally three times per week. Phylloquinone is a common and well-known substance with a publicly available chemical formula. The substance used in this trial is from Infectopharm which produces and sells the product under the trade name KA-Vit®. The study medication will be administered in the form of liquid drops under supervision at each HD session, thus ensuring very high compliance with the medication. The exact time point during the dialysis session at which the study medication will be administered is flexible,
since vitamin K1 is fat soluble and thus, is not eliminated by HD nor is its absorption affected by the dialysis procedure.

At baseline, i.e. prior to the first intake of vitamin K1, serum and plasma samples will be obtained to assess biochemical parameters, including routine laboratory parameters plus ionized calcium, phosphate, iPTH, 25-OH vitamin D3, bone alkaline phosphatase, HbA1c, total magnesium and ionized magnesium. Additional parameters that will be obtained are MGP isoforms, OC isoforms, PIVKA-II and vitamin K plasma levels.

During the treatment phase, three study visits will take place at 1, 12 and 18 months. At the 1-month study visit, MGP and OC isoforms, PIVKA-II and vitamin K plasma levels will be reassessed. In case that ucMPG levels are not substantially reduced here (20% according to [16]), the vitamin K1 dose will be doubled (10 mg as a single dose) and treatment duration extended by 1 month. After 12 months, a second MSCT scan will be performed, and MGP isoforms and vitamin K plasma levels will be measured. After 18 months, the third MSCT scan will be performed, and the same serum and plasma parameters will be assessed as at the first visit (Figure 1). Three and 5 years after treatment start, telephone interviews will record MACE and mortality of enrolled patients.

SECONDARY END POINTS

We consider the following as secondary end points:

(i) Progression of thoracic aortic calcification (absolute change in the Agatston score at the 18-month MSCT versus the baseline MSCT).

(ii) Progression of coronary artery calcification (absolute change in the volume score at the 18-month MSCT versus the baseline MSCT).

(iii) All radiological analyses mentioned above in both primary and secondary end points will also be performed at 12 months (defined as 12 months ± 4 weeks) after baseline. The 12-month observations will be used to better describe the temporal evolution of calcifications. It will also serve as an added database, which may become important should there be technical problems in individual patients (e.g. MSCT cannot be evaluated for technical reasons).

(iii) Mortality from any cause after 18 months following start of the treatment.

(vi) MACE: myocardial infarction, stroke, acute coronary syndrome, embolism, symptom-driven revascularization and death from cardiovascular cause after 18 months following start of the treatment.

The following parameters will be assessed as further outcome parameters without being a primary or secondary end point:

(i) Per cent of patients with regression of thoracic aortic calcification of at least 10% compared with baseline measure (an 18-month Agatston and volume score) as measured by MSCT.

(ii) Per cent of patients with regression of coronary artery calcification of at least 10% compared with baseline measure (an 18-month Agatston and volume score) as measured by MSCT.

(iii) All radiological analyses mentioned above in both primary and secondary end points will also be performed at 12 months (defined as 12 months ± 4 weeks) after baseline. The 12-month observations will be used to better describe the temporal evolution of calcifications. It will also serve as an added database, which may become important should there be technical problems in individual patients (e.g. MSCT cannot be evaluated for technical reasons).
(iv) Measures to assess the biochemical effect of vitamin K1 administration (including plasma levels of dp-cMGP and dp-ucMGP at 12 and 18 months).
(v) Mortality from any cause 3 and 5 years after starting the treatment.
(vi) MACE 3 and 5 years after starting the treatment.

Values obtained after starting the treatment will be compared with baseline values. MGP isoform levels will be determined by IDS Inc. (the Netherlands).

STATISTICAL CONSIDERATIONS

Randomization

If a patient is eligible to participate in the study phase and has signed the informed consent, the patient will be randomly assigned to one of the two arms. The randomization will be stratified by centre and gender. Details will be given in a randomization report, which will be kept concealed until closure of the database.

Sample size

Previous data [28] show an annual increase in thoracic aortic calcification in HD patients, measured by the volume score, of ~200 (standard deviation [SD] 235) for patients with an initial score of >100. Thus, based on a treatment time of 18 months, the expected mean increase in the volume score is ~300 (SD 235). We further assume that this increase will be 30% lower in the vitamin K1 group, resulting in an expected mean increase of ~210 (SD 235) in the volume score.

Based on the assumption that the treatment difference will occur to a similar degree in all centres, 108 patients per arm will be necessary to detect a mean difference of ~90 (SD 235) (a two-sided significance level of 5%, t-test, 80% Power, nQuery—Advisor 7.0 under Windows 7).

We further assume that comorbid conditions will result in an annual dropout rate of 25%, so that after an 18-month treatment period we expect an overall dropout rate of 37.5%. We also assume that the dropout rate will not depend on the treatment. When we correct our total sample size of 216 by a dropout rate of 37.5%, we end up with a total sample size of 348.

To include this number of patients in the trial, we will need to screen ~600 patients, given our main inclusion criterion of a volume score of >100.

Analysis of primary end points

The primary efficacy end points ‘thoracic aortic calcification’ and ‘coronary artery calcification’ are defined as absolute changes in the thoracic aortic calcification and coronary artery calcification scores measured by the volume score at the 18-month visit versus the baseline visit.

We expect that the progression, expressed as the mean absolute difference between the thoracic aortic calcification score at the 18-month visit and the thoracic aortic calcification score at baseline, will be lower in the vitamin K1 maintenance therapy group than in the standard therapy group. Consequently, the progression will be expressed as the individual change between the thoracic aortic calcification after 18 months and the calcification at baseline. The hypotheses will be tested by means of fitting an analysis of covariance (ANCOVA) model to the ‘volume score at the 18-month visit’. The model consists of the factors ‘treatment’, the stratification factor ‘centre’ and the co-variable ‘baseline volume score’, but not treatment-by-centre interaction. The test of the main factor ‘treatment’ will be performed at the significance level of 5% based on the Type II sum of squares.

In a sensitivity analysis, we will check for a possible treatment-by-centre interaction by including the corresponding interaction in our ANCOVA model.

The results on mean baseline and post-treatment scores will also be given as per cent changes. Furthermore, we will describe our analysis results by 95% confidence intervals for the treatment difference in terms of the mean change.

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CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES


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