Atherosclerosis 252 (2016) 68–74

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Circulating Des-gamma-carboxy prothrombin is not associated with cardiovascular calcification or stiffness: The Multi-Ethnic Study of Atherosclerosis (MESA)

John Danziger [a, *], Rebekah L. Young [b], Kyla M. Shea [c], Daniel A. Duprez [d], David R. Jacobs [e], Russell P. Tracy [f], Joachim H. Ix [g], Nancy S. Jenny [h], Kenneth J. Mukamal [a]

[a] Department of Medicine, Beth Israel Deaconess Medical Center, Boston, MA, United States
[b] University of Washington, Seattle, WA, United States
[c] Human Nutrition Research Center on Aging, Tufts University, Boston, MA, United States
[d] Division of Cardiology, University of Minnesota, United States
[e] School of Public Health, Division of Epidemiology & Community Health, University of Minnesota, United States
[f] College of Medicine, University of Vermont, Burlington, VT, United States
[g] Nephrology Section, Veterans Affairs San Diego Healthcare System, San Diego, CA, United States
[h] Department of Pathology, University of Vermont, Burlington, VT, United States

ARTICLE INFO

Article history:
Received 24 February 2016
Received in revised form
9 June 2016
Accepted 27 July 2016
Available online 1 August 2016

Keywords:
Vitamin K
Vascular calcification
Vascular stiffness
Medial calcification
Phylloquinone
Matrix Gla protein

ABSTRACT

Background and aims: Vitamin K-dependent protein (VKDP) activity may have a role in preventing cardiovascular calcification, but has not previously been studied in large, generally healthy populations.
Methods: Using an elevated ankle-brachial index (ABI) as a measure of medial vascular calcification, we performed a case-cohort analysis within the Multi-Ethnic Study of Atherosclerosis, measuring Des-gamma-carboxy prothrombin (DCP) to estimate VKDP activity. In secondary analyses of the weighted subcohort, we examined the cross-sectional associations between DCP and prevalent vascular calcification of the coronary vessels, aortic and mitral valves, and aortic wall, and with vascular stiffness.
Results: In adjusted analysis, cases (n = 104) had 0.21 ng/ml (−0.94–0.52) lower DCP concentrations than the subcohort (n = 613). Furthermore, amongst the 717 participants in the weighted cohort, VKDP activity was not associated with coronary artery, mitral valve, aortic valve or aortic wall calcification, nor was it associated with vascular stiffness.
Conclusions: Our negative results do not support a role of circulating VKDP activity in cardiovascular calcification in community-dwelling adults.

1. Introduction

VKDPs are a large class of proteins unique in their dependence on post-translational modification to achieve biologic activity, a complex process whereby glutamate (Glu) residues undergo carboxylation to the γ-carboxyglutamate (Gla) form, requiring enzymatic activity and vitamin K [1]. Emerging data suggest that VKDPs may play an important role in vascular biology. Localizing to the vascular wall, VKDPs such as matrix Gla protein [2–4] and Gas-6, are natural calcification inhibitors, providing local defense against vascular calcification [5]. Animal models suggest that VKDP inactivity causes a specific type of calcification, involving the medial wall with relative intimal sparing, otherwise known as Monckeberg's sclerosis [6,7]. Observational data have linked vitamin K insufficiency to an increased risk of vascular calcification [8,9]. A randomized study has suggested that dietary vitamin K supplementation may retard coronary calcification progression [10], with further interventional studies ongoing [11].

In addition to dietary vitamin K intake, other factors affect VKDP activity, including enzyme polymorphisms [12–14], renal function [15], hepatitis [16], malignancy [17], and warfarin exposure [18].

Abbreviations: VKDP, Vitamin K dependent protein; MESA, Multi Ethnic Study of Atherosclerosis; ABI, ankle brachial index; DCP, Des-gamma-carboxy prothrombin; MGP, matrix Gla protein; Gas-6, growth arrest specific factor 6; LDL, low density lipoprotein; HDL, high density lipoprotein.

* Corresponding author. Division of Nephrology, Beth Israel Deaconess Medical Center, 185 Pilgrim Road, Boston, MA 02215, United States.
E-mail address: jdanzige@bidmc.harvard.edu (J. Danziger).

http://dx.doi.org/10.1016/j.atherosclerosis.2016.07.924
0021-9150/

VKDP activity can be determined by measuring the under-carboxylated form of one of several VKDPs, including MGP, osteocalcin, and prothrombin [19–21]. Prothrombin is produced by the liver, and undergoes rapid carboxylation to its circulating form; its under-carboxylated form, Des-gamma-carboxy prothrombin (DCP), indicates VKDP inactivity [22]. The biologic function of DCP is not known. To date, scant data exist on whether VKDP activity, independent of nutritional vitamin K stores, is associated with cardiovascular calcification in community populations [23].

Using the Multi-Ethnic Study of Atherosclerosis (MESA), a large, well-phenotyped study of generally healthy adults from six field centers across the U.S., we examined whether VKDP activity is cross-sectionally associated with either vascular or valvular calcification, or vascular stiffness. We conducted a case-cohort study of vascular stiffness, characterized by an elevated ankle-brachial index (ABI) $\geq$ 1.4 [24]. In addition, using a weighted analysis of cases and the subcohort, we explored the cross-sectional associations of VKDP activity with calcification of the coronaries, cardiac valves, and aorta, along with other measures of vascular stiffness.

2. Materials and methods

2.1. Participants

Details of the study design and protocol for MESA have been published [25]. In brief, between July 2000 and August 2002, 6814 community-dwelling adults, free from clinically apparent cardiovascular disease, between the ages of 45–84 years, were recruited from 6 diverse communities and participated in the baseline examination. The cohort is comprised of 38% Caucasian (N = 2622), 28% African-American (N = 1893), 22% Hispanic (N = 1496) and 12% Chinese (N = 803) participants. Individuals underwent extensive clinical, laboratory, and radiographic examination as part of the baseline examination. Measures of subclinical atherosclerosis included ABI measurements, coronary and thoracic computerized tomography scan imaging, cardiac magnetic resonance imaging for evaluation of left ventricular mass and function, and measures of vascular stiffness. The institutional review boards at all participating centers approved the study, and all participants gave informed consent.

We used a case-cohort design, randomly sampling from a previously drawn random subcohort of MESA participants with serum vitamin K1 measurements [8]. Our case-cohort study defined cases as participants who had an ABI $\geq$ 1.4. After excluding participants taking warfarin (n = 24) and participants who were part of another random subcohort in MESA that were not eligible for additional use of stored biological samples (n = 995), 104 participants were identified as cases, as defined by an ABI $\geq$1.4. The minimum ratio of either the left or right ankle blood pressure to brachial (arm) blood pressure was used [26]. The subcohort (n = 616) was a completely random subsample drawn without replacement from the 780 members who underwent vitamin K1 measurement (including 11 participants who were also cases) and who were not excluded based on the criteria above (Supplemental Fig. 1).

2.2. VKDP activity

The primary exposure was VKDP, as determined by DCP concentrations, using a commercially available ELISA kit (Asserachrom DCP-II, Stago, France) that uses murine monoclonal antibody to detect under-carboxylated prothrombin. Lower DCP concentrations suggest greater VKDP activity. Although no formal threshold for "normal" DCP concentrations exists, previous studies have suggested a threshold of 2 ng/ml is responsive to high dietary vitamin K intake [27]. The detectable range for this assay was 0.335–207 ng/ml. Based on 4 controls, the intra-assay CVs were 6.5%, 16.1%, 5.2%, and 12.2%, and the inter-assay CV were 10.2%, 32.3%, 9.1%, and 12.5%.

2.3. Vascular calcification and stiffness

Coronary artery calcium, aortic valve calcium, mitral valve calcium, and descending thoracic aorta calcium were assessed using either a cardiac-gated electron beam computed tomography scanner or a multi-detector computed tomography system [28,29]. All participants were scanned twice. The mean phantom-adjusted Agatston score was used in all analyses [30]. To measure vascular stiffness, we used pulse pressure, and small (SAE) and large artery elasticity (LAE) indices [31–33]. Pulse pressure was defined as resting seated systolic blood pressure minus diastolic blood pressure acquired in the resting position (using aDinamap® automated blood pressure device). Three sequential measures were obtained and the average of the second and third measurements was recorded. To estimate the SAE and LAE indices, the HDI PulseWave CR-2000 Research CardioVascular Profiling Instrument (HDI, www.hdi-pulsewave.com) was used to acquire and analyze pulse waveforms from radial artery tonometry. The pulse contour analysis technique incorporates pressure fluctuations and describes changes in artery distention pressures throughout the cardiac cycle as a measure of compliance, as described [32,34].

2.4. Covariates

Age, gender, race/ethnicity (white, Chinese, African-American, or Hispanic), high school graduation, household income, smoking history, current alcohol use, and physical activity were self-reported through standard questionnaires. Participants' medications were inventoried during the exam. Body mass index was calculated from height and weight measurements obtained without shoes. Diabetes was defined by either a fasting glucose $\geq$126 mg/dl or the use of either oral hypoglycemic medications or insulin. Fasting blood was collected and stored at −70 until needed for the appropriate assays. High density lipoprotein (HDL) cholesterol was measured using the cholesterol oxidase cholesterol method (Roche Diagnostics), and low density lipoprotein (LDL) was calculated using the Friedewald equation. The estimated glomerular filtration rate (eGFR) was calculated by using the Chronic Kidney Disease Epidemiology Collaboration formula [35] and serum creatinine measurements. Urine albumin and creatinine were measures in a single morning sample by nephelometry and the rate Jaffe equations, and expressed as albumin-creatinine ratio (ACR) in mg/g.

Nutritional vitamin K status was determined by measurement of vitamin K1, (serum phylloquinone) as measured by reversed-phase HPLC followed by fluorometic detection [8]. In addition, vitamin K2 (serum dihydrophylloquinone), synthesized from hydrogenated trans saturated fats, was also measured.

2.5. Statistical analysis

We present baseline characteristics stratified by case-cohort status and by DCP quartiles, along with the distribution of DCP concentrations. Sequential multivariate linear models were used to describe the association between case-control status and DCP concentrations. Model 1 adjusted for age (years), gender and race/ethnicity (Black, Hispanic, Chinese, white). Model 2 adjusted for Model 1 plus body mass index (BMI), cigarette smoking (never, former, current), intentional physical activity (MET-min/day), current alcohol use, high school graduation, diabetes, systolic blood pressure (SBP), LDL and HDL cholesterol, triglycerides, high-