Relation of circulating matrix Gla-protein and anticoagulation status in patients with aortic valve calcification

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Summary
Matrix-Gla Protein (MGP) is a vitamin K-dependent protein acting as a local inhibitor of vascular calcification. Vitamin K-antagonists (oral anticoagulant; OAC) inhibit the activation of MGP by blocking vitamin K-metabolism. The aim of this study was to investigate the effect of long-term OAC treatment on circulating MGP levels in humans and on MGP expression in mice. Additionally, we tested the association between circulating inactive MGP (ucMGP) levels and the presence and severity of AVC in patients with aortic valve disease (AVD). We analysed circulating ucMGP levels in 191 consecutive patients with echocardiographically proven calcific AVD and 35 control subjects. The extent of AVC in the patients was assessed by multislice spiral computed tomography. Circulating ucMGP levels were significantly lower in patients with AVD (348.6 ± 123.1 nM) compared to the control group (571.6 ± 153.9 nM, p < 0.001). Testing the effect of coumarin in mice revealed that also the mRNA expression of MGP in the aorta was downregulated. Multifactorial analysis revealed a significant effect of glomerular filtration rate and long-term OAC therapy on circulating ucMGP levels in the patient group. Subsequently, patients on long-term OAC had significantly increased AVC scores. In conclusion, patients with calcific AVD had significantly lower levels of circulating ucMGP as compared to a reference population, free of coronary and valvular calcifications. In addition, our data suggest that OAC treatment may decrease local expression of MGP, resulting in decreased circulating MGP levels and subsequently increased aortic valve calcifications as an adverse side effect.

Keywords
Oral anticoagulation, aortic valve calcification, protein function, imaging, matrix Gla protein

Blood Coagulation, Fibrinolysis and Cellular Haemostasis

Introduction
The activity of vitamin K-dependent proteins is strictly dependent on the presence of the vitamin K (1). Vitamin K is the cofactor in the posttranslational conversion of specific protein bound glutamate (Glu) residues into γ-carboxyglutamate (Gla) residues. One of these vitamin K-dependent proteins is matrix Gla-protein (MGP), a potent local inhibitor of calcification, abundantly synthesised by chondrocytes and vascular smooth muscle cells (2, 3). Vitamin K deficiency leads to uncarboxylated MGP and thus impairment of its biological function (4, 5). MGP plays a key role in the inhibition of tissue calcification, which was demonstrated in MGP-deficient mice. These animals all developed excessive medial artery calcifications and subsequent ruptures of the heavily calcified vasculature leading to death within six to eight weeks after birth (6).

Previous studies suggest that oral anticoagulant (OAC) treatment with vitamin K antagonists not only affects the carboxylation of vitamin K-dependent clotting factors, but also affects the action of the extra-hepatic protein MGP (7). Price et al showed in rats that after three to four weeks of treatment with warfarin, arterial calcifications were present around the elastic lamellae of smooth muscle cells.
the tunica media (7). In two independent studies it was shown that patients on OAC treatment had increased coronary artery and aortic valve calcification (8, 9).

Patients with moderate to severe valvular and coronary artery calcifications were found to have an unfavorable prognosis compared to patients with no or mild calcification (10, 11). Therefore, the measurement of biomarkers which can predict or reflect disease and/or its progression becomes of substantial importance. Recently, the measurement of circulating ucMGP levels as a biomarker was described (12–14). It was demonstrated that this assay identified a wide range of patient populations prone to develop arterial calcification. In this study we investigated the possible role of circulating MGP as biomarker for valvular calcification in patients with calcific aortic valve disease. Secondly, we aimed to determine the impact of long-term OAC treatment in patients with aortic valve disease on both calcification and serum ucMGP levels. Finally, we investigated potential mechanisms leading to decreased levels of ucMGP in patients employing a murine model of warfarin-induced calcification.

Methods

Subjects
The study group included 191 consecutive patients [135 (71%) men, mean age 71 ± 9 years, range: 39–89 years] with echocardiographically proven calcific aortic valve disease. These patients were recruited consecutively at the echocardiography outpatient department or at the inpatient cardiology department of the University Hospital Aachen between May 2004 and March 2006. Both patients with aortic sclerosis as well as patients with mild, moderate or severe aortic valve stenosis were included. Twenty-seven of these patients (14%) received long-term OAC treatment (> 48 months) because of atrial fibrillation (n=19, 70%), thrombosis or pulmonary embolism (n=3, 11%), reduced left ventricular function (n=4, 15%) or prior stroke (n=1, 4%). The exclusion criteria were increased serum calcium (calcium > 2.6 mM) or end-stage renal disease (glomerular filtration rate [GFR] < 15 ml/min according to the MDRD formula [15]). Four patients eligible for inclusion refused to give their consent. After inclusion blood samples were taken to determine ucMGP serum levels. Moreover, a non-enhanced MSCT examination was performed within two days after echocardiography in all patients of the study cohort.

Data concerning patient history, medication, coronary risk factors and routine laboratory parameters were obtained by the patient interview and chart review. Glomerular filtration rate was calculated based on the modified MDRD formula (15). Cardiovascular risk factors were assessed from the patient chart including nicotine abuse, hypertension (use of antihypertensive medication or blood pressure at rest > 140/90 mmHg), diabetes mellitus (use of insulin or oral antidiabetic agents or fasting serum glucose > 130 mg/dl), hypercholesterolemia (total fasting serum cholesterol > 200 mg/dl or use of cholesterol-lowering medication) and obesity (body mass index [BMI; body weight / body length²] > 25 kg/m²). Additionally, the presence of ischemic heart disease as defined by a history of myocardial infarction and/or coronary artery stenosis (> 50%) on coronary angiography, was evaluated.

In addition, a reference group of 35 control subjects (5 (14%) men, mean age 56 ± 10 years, range: 31–70 years) was included in the study. These control subjects had no history of aortic valve disease and coronary artery disease. In all control subjects a non-enhanced MSCT examination was performed for calcium scoring. Prerequisite for serving as a control subject was the absence of any significant amount of coronary and valvular calcification (Agatston score ≤ 10).

Informed consent was obtained from each participant prior to the investigation and the study was approved by local Ethics Committee.

ucMGP measurement
After inclusion, blood samples were taken to determine ucMGP serum levels. Blood was collected from all participants by venipuncture (10 ml) in serum tubes and stored for 30 minutes (min) at room temperature before centrifugation (15 min 1,580 × g). Serum was sub-sampled in 250 µl aliquots and frozen at –80 ºC until testing.

The method for ucMGP measurement was a competitive enzymelinked immunosorbent assay (ELISA) using a monoclonal antibody (mAb-ucMGP) which was raised against the uncarboxylated human MGP Gla-domain (MGP sequence 35–49; VitaK BV, Maastricht, The Netherlands) and screened for conformational affinity toward full-length synthetic cMGP (5 Gla) or synthetic ucMGP (5 Gla) (12, 14). In brief, mAb-ucMGP was coupled to the microtiter plate. Serum sample or standard was supplemented with tracer (biotinylated MGP35–54) and transferred to the microtiter plate and incubated overnight at 4°C. The ucMGP concentration was calculated with the aid of a calibration curve of synthetic full length ucMGP. The intra-assay and inter-assay coefficients of variation (CV) of the ucMGP assay were calculated to be 8.9 % and 11.4 %.

Imaging procedures
In all patients of the study population echocardiography was performed using a commercially available ultrasonographic system (GE Vingmed, Vivid 7, Horten, Norway) by one experienced echocardiographer. Aortic valve sclerosis was defined as focal area of increased echogenicity and thickening of the aortic valve leaflets with a transaortic flow velocity < 2.5 m/s on transthoracic echocardiography, using the criteria of Otto et al (16, 17). In case of a transaortic flow velocity of ≥ 2.5 m/s and ≤ 3.0 m/s patients were classified as mild aortic stenosis; those with a transaortic flow velocity of ≥ 3.0 m/s and ≤ 4.0 m/s as moderate and patients with a transaortic flow velocity > 4.0 m/s as severe aortic stenosis (18). In patients with left ventricular dysfunction (n=18, 9%), defined as left ventricular ejection fraction < 55%, aortic valve area was calculated with the continuity equation. Severity of aortic valve stenosis was graded according to the ACC/AHA guidelines (18).

All MSCT examinations were performed with a 16-slice MSCT scanner (Sensation 16, Siemens, Forchheim, Germany; collimation 12 x 0.75 mm, tube rotation time 420 ms, table feed 3.4 mm/rotation, tube voltage 120 kV with an effective tube current time product of 150 mAAs, using a standardised imaging protocol with retrospective electracardiographic (ECG) gating. The average heart rate of the patients and control subjects was
66.4 ± 6.1 beats per min. Axial images were reconstructed at 60% of the RR interval as recommended for coronary calcium screening with an effective slice thickness of 3 mm and a reconstruction increment of 2 mm using a dedicated convolution kernel (B35f) (19). The field of view was 180 x 180 mm² with a 512² matrix.

MSCT images were assessed in a consensus reading by an experienced radiologist and an experienced cardiologist. Both readers were blinded to all patient data. Image analysis was performed on a separate computer workstation (Leonardo, Siemens, Forchheim, Germany) equipped with a dedicated software tool for calcium scoring (Calcium Scoring CT, Siemens). For quantitative assessment of aortic valve and coronary calcification, respectively, the Agatston score was calculated with a detection threshold of 130 Hounsfield units (20). A detailed description of calcification assessment is described previously (21).

Animals and diet
To investigate aortic MGP regulation during warfarin treatment, we transferred the established rat model of warfarin mediated vascular calcification (22) into DBA/2 mice. The local animal welfare committee approved the animal study protocol. Ten-week-old mice were purchased from Taconic (Tornbjerg, Denmark) and maintained in a temperature-controlled room on a 12-hour day/night cycle. Food and water were given ad libitum. To assure steady warfarin and vitamin K intake in the warfarin treatment group (n=5) vitamin K-deficient food (Hope Farms, Woerden, The Netherlands) was supplemented with warfarin (final concentration 3 mg/g food) and spiked with vitamin K1 (final concentration 1.5 mg/g food). Using this approach, extra-hepatic Glu-protein synthesis could be blocked and fatal bleeding diathesis could efficiently be avoided. Control mice (n=5) received standard chow (Altromin 1324; Altromin GmbH, Lage, Germany). After four weeks of treatment the mice were sacrificed and the aortas were removed.

RNA isolation and absolute quantitative RT real-time PCR
RNA extraction and real-time PCR was performed as described previously (23). Briefly, RNA was extracted according to RNA-later® and RNAeasy® protocols (QIAGEN, Hilden, Germany). Integrity and amount of RNA were measured by capillary electrophoresis (Agilent Bioanalyzer 2100, Agilent Technologies, Böblingen, Germany). Reverse Transcription and real-time PCR were performed on 100 ng RNA using a commercial RT-PCR kit (Eurogentech, Cologne, Germany) and the ABI 7700 sequence detection system (PE, Applied Biosystems, Inc., Foster City, CA, USA). MGP specific primers were derived from Ensembl entry ENSEMBL00000032342: sense: GCAGAGGTGGCAGC-GAAAAGCAGATC-TAMRA, antisense: AGCTGGTGGGCTTCCC, probe: FAM-AGAGCCGAGATGG-GAAGCTTGTCATC-TAMRA, to yield an amplicon length of 104 bp. By co-amplification of known quantities of pGEM-T plasmids (Promega, Madison, WI, USA) containing the cloned target genes (MGP, OPN, GAPDH). Finally, results were normalized to mRNA levels of the housekeeping gene GAPDH.

Statistical analysis
Continuous variables are expressed as mean values ± standard deviation (SD), categorical data are presented as frequencies. The unpaired t-test was used for comparisons of continuous variables and the chi-square test was used for comparisons of categorical variables between patients with aortic valve calcification (study group) and reference subjects (control group).

In the control group one-factorial analysis of variance (ANOVA) and linear regression analysis was conducted in order to explore the effects of several cardiovascular risk factors as well as calculated GFR on serum ucMGP levels.

In the study group multifactorial analysis of covariance (ANCOVA) was used to explore the effects of long-term anticoagulation therapy (≥48 months), the covariables sex, age, body mass index, diabetes, smoking status, hypertension, hypercholesterolemia, GFR as well as the presence of drugs (beta-blockers, angiotensin converting enzyme [ACE] inhibitors, diuretics, cholesterol lowering drugs, thyroid hormones, antidepressants) on serum ucMGP levels and aortic valve calcification (AVC) scores, respectively. Due to the comparatively large numbers of independent factors, either simple linear regression (in case of

| Table 1: Clinical and laboratory characteristics of the study and control group. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
|                 | Study group     | Control group   | P value*        |
| Age (years)     | 71 ± 9          | 56 ± 10         | <0.01           |
| Coronary artery disease, n (%) | 115 (60) | 5 (0) | <0.01 |
| Cardiovascular risk factors | | | |
| Body mass index, kg/m² | 27.6 ± 4.7 | 25.3 ± 3.6 | <0.01 |
| Hypertension, n (%) | 140 (73) | 12 (34) | <0.01 |
| Hypercholesterolemia, n (%) | 132 (69) | 5 (14) | <0.01 |
| Smoking, n (%) | 75 (39) | 4 (11) | <0.01 |
| Diabetes mellitus, n (%) | 34 (18) | 2 (6) | 0.05 |
| Laboratory chemical analysis | | | |
| Calcium, mg/dl | 2.36 ± 0.14 | 2.37 ± 0.16 | 0.55 |
| Glomerular filtration rate, ml/min | 65.4 ± 21.1 | 68.3 ± 21.6 | 0.50 |
| Total cholesterol, mg/dl | 194 ± 56 | 202 ± 36 | 0.25 |
| LDL-cholesterol, mg/dl | 131 ± 48 | 105 ± 32 | <0.01 |
| C-reactive protein, mg/l | 13.6 ± 16.9 | 9.0 ± 7.1 | 0.01 |

(mean ± standard deviation, if not specified separately. * analyzed solely in an explorative manner. LDL, low-density lipoprotein.)
the continuous covariables age, body mass index and GFR) or one-factorial analysis of variance (ANOVA) in case of binary factors were conducted firstly in order to select relevant factors possibly influencing ucMGP levels and Agatston AVC scores, respectively (factors with a p-value of p ≤ 0.2); only these factors were examined in the final multifactorial ANCOVA models. Statistical significance of the independent factors used in these models was assessed by global F-tests. Additionally, in the study group the degree of linear association between ucMGP levels and AVC scores was investigated by computing the Pearson’s correlation coefficient.

The global significance level for all statistical tests conducted was chosen to α=0.05 and adjusted for multiple testing using the Bonferroni method. Thus, a p-value of p ≤ 0.0167 was interpreted as statistically significant test result. Results of all statistical tests carried out within the control group are interpreted solely in an explorative manner.

Statistical analysis was performed with the use of SAS statistical analysis software package (version 9.1, SAS Institute, Cary, NC, USA).

Results

Subject characteristics
The clinical and laboratory characteristics of both the patient population and control group are shown in Table 1. Patients with aortic valve disease were older and cardiovascular risk factors were more prevalent (Table 1). Although nine patients had chronic kidney disease (CKD) stage 4 and none of the control subjects had chronic kidney disease stage 4, there was no significant difference in mean GFR levels between the patient and control group (Table 1).

None of the subjects in the control group had a history of coronary artery disease. The mean coronary Agatston score determined by MSCT in these subjects was 1.16 ± 2.84 (range: 0–10.0); no valvular calcifications were found. In the patient population, 71 patients demonstrated aortic sclerosis (37%; AVC 1), 57 patients presented with mild aortic valve stenosis (30%; AVC 2), 36 patients revealed moderate aortic valve stenosis (19%; AVC 3) and 27 patients showed severe aortic stenosis (14%; AVC 4) according to the peak transaortic flow velocity determined by echocardiography. The mean of the Agatston AVC score for the entire study group was 1384 ± 1453 (range: 0.6–6973).

Serum ucMGP levels
Serum ucMGP-levels were significantly lower in the study group (348.6 ± 123.1 nM) than in the reference population (571.6 ± 153.9 nM, p < 0.001; Fig. 1). In the study group, no differences in serum ucMGP levels between patients with coronary artery disease (n=115, 346 ± 109 nM) versus patients without coronary artery disease (n=76, 353 ± 143) were found. No association between serum ucMGP levels and the severity of aortic valve disease was observed when comparing the control group with the patient population subdivided according to the severity of aortic stenosis (AVC 1 through 4; Fig. 1). In addition, there was no correlation between serum ucMGP levels and Agatston AVC scores in the study group (r=0.01).

Figure 1: Bar graph demonstrating serum ucMGP levels in patients with different severity degrees of aortic stenosis (n=191) as well as in the reference population (n=35). * = p<0.001 versus all AVC groups. AVC 1= patients with aortic sclerosis (n=71); AVC 2= patients with mild aortic valve stenosis (n=57); AVC 3= patients with moderate aortic valve stenosis (n=31); AVC 4= patients with severe aortic valve stenosis (n=27).

Table 2: Multifactorial analysis of covariance of clinical patient characteristics with respect to ucMGP levels.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>DF</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>1</td>
<td>3.57</td>
<td>0.588</td>
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<tr>
<td>Body mass index</td>
<td>1</td>
<td>1.83</td>
<td>0.088</td>
</tr>
<tr>
<td>Long-term anticoagulation</td>
<td>2</td>
<td>4.54</td>
<td>0.014*</td>
</tr>
<tr>
<td>Glomerular filtration rate</td>
<td>1</td>
<td>9.99</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Cholesterol-lowering drugs</td>
<td>1</td>
<td>0.20</td>
<td>0.7831</td>
</tr>
</tbody>
</table>

DF, degrees of freedom; degrees of freedom for error 129; F-value, value of test statistic of global F-test. * Statistically significant test result after Bonferroni adjustment.

Table 3: Multifactorial analysis of covariance of clinical patient characteristics with respect to AVC scores.

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>DF</th>
<th>F-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
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<td>3.23</td>
<td>0.074</td>
</tr>
<tr>
<td>Long-term anticoagulation</td>
<td>2</td>
<td>5.66</td>
<td>0.004*</td>
</tr>
<tr>
<td>Hypertension</td>
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<td>0.79</td>
<td>0.376</td>
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<tr>
<td>Hypercholesterolemia</td>
<td>1</td>
<td>0.62</td>
<td>0.432</td>
</tr>
<tr>
<td>Cholesterol lowering drugs</td>
<td>1</td>
<td>0.44</td>
<td>0.507</td>
</tr>
<tr>
<td>Betablockers</td>
<td>1</td>
<td>2.45</td>
<td>0.120</td>
</tr>
<tr>
<td>ACE inhibitors</td>
<td>1</td>
<td>6.59</td>
<td>0.011*</td>
</tr>
<tr>
<td>Thyroid hormones</td>
<td>1</td>
<td>2.44</td>
<td>0.120</td>
</tr>
</tbody>
</table>

DF, degrees of freedom; degrees of freedom for error 129; F-value, value of test statistic of global F-test. * Statistically significant test result after Bonferroni adjustment.
Anticoagulation status, risk factors and ucMGP levels

In the control group, there was no significant effect of age, sex, BMI, smoking status, diabetes, hypertension, hypercholesterolemia and calculated GFR on ucMGP levels. In the patient group, age, BMI, calculated GFR, long-term anticoagulation status as well as the presence of cholesterol lowering drugs were identified as relevant factors possibly influencing ucMGP levels. These variables were examined in the ANCOVA model showing that only calculated GFR and long-term anticoagulation status were significantly associated with low serum ucMGP levels (Table 2). In addition, a correlation between international normalised ratio (INR) values and ucMGP levels ($r = -0.64$, $p=0.001$) were observed in patients receiving long term anticoagulant treatment (mean INR $2.37 \pm 0.50$).

Concerning renal insufficiency in the patient population, patients with CKD stage 4 ($n=9$, GFR between 15–30 ml/min) showed significantly lower ucMGP levels than patients with normal to moderately impaired renal function ($n=182$, GFR > 30 ml/min, CKD 1–3; Fig. 2). In addition, patients with long-term anticoagulation ($n=27$) demonstrated lower serum ucMGP levels compared to patients without anticoagulation ($n=142$; Fig. 3). There were no significant differences in mean GFR between patients with long-term anticoagulation (GFR $64.4 \pm 23.2$ ml/min) compared to patients without anticoagulation therapy (GFR $65.6 \pm 21.2$, $p=0.80$).

Anticoagulation status, risk factors and AVC scores

The variables age, anticoagulation status, hypertension, hypercholesterolemia and the presence of cholesterol lowering drugs, beta-blockers, ACE inhibitors and thyroid hormones were identified as relevant factors possibly influencing AVC scores in the study population. No impact of GFR was found on AVC scores. In the ANCOVA model, only the long-term use of anticoagulation and the use of ACE inhibitors remained to be significantly associated with AVC scores in the study population (Table 3). Patients with ACE inhibitor use showed lower AVC scores ($n=138$, $1,063 \pm 1,179$) than patients without ACE inhibitor treatment ($n=53$, $1,985 \pm 1,819$, $p=0.001$).

Warfarin influences MGP and osteopontin mRNA expression in mice

Four weeks of warfarin administration induced profound media calcification in all mice originating from the elastic lamellae of the aorta as previously described in rats (data not shown). Using realtime PCR analysis, we detected a down-regulation of MGP mRNA levels by 50% in calcifying aortas of warfarin treated...
mice compared to controls (Fig. 4A). In contrast, aortic osteopontin expression, a marker of osteoblast-like cell differentiation was increased by threefold (Fig. 4B). The inversely arranged regulation of MGP and osteopontin mRNA expression may indicate a phenotype switch of vascular smooth muscle cells during experimental warfarin administration towards osteoblast-like phenotype.

Discussion

We performed a cross-sectional study in patients with calcific aortic valve disease, in whom valve calcification quantification allowed a comprehensive insight into the association of valvular calcification with long-term OAC use and circulating ucMGP levels. Three issues warrant special attention. First, patients with calcific aortic valve disease have significantly decreased levels of circulating ucMGP compared to subjects without valvular and coronary calcifications. Although the control subjects were younger, the impact of age on ucMGP levels was neglectable, as was shown previously (12). There was no correlation between serum MGP levels and the severity of aortic valve disease. Second, our data suggest that long-term OAC treatment and severely impaired renal function (> CKD 3) are associated with low circulating ucMGP levels. Thirdly, our results provide further evidence between an association of long-term OAC treatment and the quantity of aortic valve calcification (Table 4). In the present study, multivariate analysis revealed that GFR was associated with low circulating ucMGP levels in the patient population. Moreover, patients with severely impaired renal function (CKD stage 4) had significantly lower ucMGP levels compared to patients with normal to moderately impaired renal function (CKD stage 1 to 3). This is consistent with a previous study showing significantly lower ucMGP levels in dialysis patients as compared to controls (ucMGP 181 ± 91 nM vs. 504 ± 98 nM) (21). The low ucMGP levels in these patients could not be explained by filtration during haemodialysis since ucMGP was not detectable in the filtrate and levels did not change significantly between pre- and post-dialysis measurements (personal communications). Additionally, the even lower ucMGP levels in hemodialysis patients with calcific uraemic arteriolopathy (calciphylaxis), a widespread arteriolar calcification process, also strongly point towards a calcification-induced reduction of ucMGP rather than dialysis-induced reduction (12). We hypothesise therefore that the low ucMGP levels are the result of consumption of MGP in the vascular wall with a diminished spill-over into the circulation.

The association of long-term OAC treatment with decreased MGP levels as well as with increased aortic valve calcification is in agreement with previous in-vivo and in-vitro studies (7–9, 24). The warfarin-induced calcification model in animals is based on the complete blockage of the recycling enzyme in the vitamin K cycle, the KO-reductase (VKOR). Price et al. showed that warfarin treatment of rats resulted in calcification of the aortic heart valve and elastic media (elastocalcinosis) of major arteries with a parallel 2.5– to three-fold decrease of their serum MGP levels (7, 24). Using this warfarin induced calcification model in mice, we here provide an additional explanation for the

What is known about this topic?

- Matrix-Gla Protein (MGP) is a vitamin K-dependent inhibitor of vascular calcification.
- Vitamin-K antagonists (oral anticoagulants) inhibit the activation of MGP by blocking vitamin-K metabolism resulting in inactive MGP (ucMGP) species.
- Long-term oral anticoagulation treatment was associated with increased aortic valve calcifications in humans.

What does this paper add?

- Patients with calcific aortic valve disease had significantly lower levels of circulating ucMGP as compared to a reference population free of coronary and valvular calcification.
- The results of this study suggest that oral anticoagulant treatment and severely impaired renal function (> CKD 3) are associated with decreased ucMGP levels.
- Oral anticoagulation therapy may decrease local expression of MGP resulting in decreased circulating ucMGP levels and subsequently increased aortic valve calcifications as an adverse side effect.
lowered circulating MGP levels. Warfarin treatment induced a 50% decrease in mRNA levels of MGP, and in addition a three-fold increase in osteopontin, a marker of osteoblast-like cell differentiation. It is known that MGP is an inhibitor of bone morphogenetic protein-2 (BMP2), an inducer of bone (25). At the onset of anticoagulation, total MGP synthesis remains constant, but during the initial stage the balance between carboxylated and undercarboxylated MGP shifts in favor of the latter. By inhibiting MGP function, which is thus unable to inhibit BMP2 function, the phenotype of vascular smooth muscle cells can differentiate to a bone cell-like phenotype. As a consequence, MGP levels are decreased, whereas bone markers such as osteopontin are increased. Furthermore, uncarboxylated Gla-proteins are secreted less efficiently from the cell, possibly due to a control system identifying and removing incompletely carboxylated proteins. In the next phase, if OAC treatment is prolonged, vascular calcification will be induced due to the absence of sufficient active MGP, resulting in an entrapment of MGP in the vasculature and thus in decreased amounts of MGP set free in the circulation. The accumulation of ucMGP in intimal and medial calcifications has been reported in several studies (24, 26). This needs to be corroborated by extended longitudinal studies, however. Our findings are also consistent with retrospective studies showing that OAC treatment is associated with increased valvular and coronary calcification possibly due to incomplete vitamin K-dependent γ-carboxylation of MGP (8, 9).

Treatment with long-term OAC may also negatively interfere with other Gla-proteins such as Bone-Gla Protein (BGP, osteocalcin). In this respect, a large retrospective data-base study revealed an increased fracture risk in men on long-term OAC treatment (> 12 months) (27). Therefore, the possible association of long-term OAC treatment and induction of vascular and valvular calcification may imply to prescribe OAC cautiously in all disease conditions in which arteriosclerosis is the predominant finding. However, prospective randomised trials are necessary to address and clarify this possible association.

In this study, we also analysed the relationship between medication use and circulating ucMGP levels and arterial valve calcification. In contrast to the conductive impact of long-term anticoagulation, the intake of angiotensin-converting enzyme (ACE) inhibitors was associated with a decreased arterial valve calcification. In previous studies an involvement of the renin-angiotensin system in the pathogenesis of aortic valve calcification, i.e. the presence of ACE and the induction of local angiotensin II-producing systems in stenotic aortic valves has been demonstrated (28). In addition, ACE inhibitors have been shown to slow-down aortic valve calcium accumulation (29). However, there are no data that have been proven to delay the haemodynamic progression of aortic stenosis (30).

The limitations of this study comprise the cross-sectional nature which precludes the definition of a clear cause-and-effect relation between the factors investigated. Additionally, the lack of immunohistochemical analysis of MGP accumulation in complimentary vascular and aortic valve is missing and needs to be evaluated in future studies. Also relevant coronary and valvular calcifications assessed by cardiac MSCT for the control group were absent, therefore we choose for a younger control group. In a previous study, however, age did not influence MGP levels (12). In addition, in this study multivariate analysis did not reveal an effect of age on MGP levels in the control group.

In conclusion, our results suggest that OAC treatment, which is widely used for antithrombotic therapy, may induce as an adverse side effect decreased MGP serum levels and increased aortic valve calcifications. Longitudinal studies are needed to evaluate if ucMGP serum levels may become a suitable biomarker for the progression of aortic valve calcification.

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