Gülhane Tıp Derg 2011; 53: 26-30

Does warfarin therapy have a role in coronary calcification?

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SUMMARY

Some reports have revealed that warfarin inhibits the matrix carboxyglutamic acid protein (MGP), which inhibits calcification. Treatment of rats with warfarin at doses that inhibit the carboxylation of MGP causes rapid calcification of elastic lamellae of arteries and of aortic heart valves and increased expression of MGP-mRNA in the calcifying artery. Therefore warfarin use might result in increased calcification of vessel wall. Our aim was to investigate whether warfarin causes excess calcification in coronary arteries. A total of 39 patients (with a mean age of 54±15 years) who underwent prosthetic heart valve surgery 66±46 months ago were enrolled into the study. The control subjects (n=28) were selected from those people without any medical problem with a mean age of 52±11 years. Multidetector computed tomography was used to identify the calcium score of the coronary arteries. We found that coronary calcium score was similar between the study and control groups. In addition, the length of warfarin use was not correlated with the calcium score in the patient group. Male gender, hypertension, and LDL-C, but not warfarin usage were independent predictors for coronary calcification. The results of our study imply that warfarin has no effect on coronary calcification at least in middle aged adult humans.

Key words: *Coronary calcification, multidetector computerized tomography, warfarin*

ÖZET

Warfarin tedavisinin koroner kalsifikasyonda rolü var mıdır?

Warfarinin kalsifikasyonu inhibe eden matriks karboksiglutamik asid proteinini (MGP) inhibe ettiği bildirilmektedir. MGP karboksilasyonunu inhibe eden dozlarda K vitamini antagonisti olan warfarin verilen ratlarda arterlerin elastik laminalarında ve aort kapağında hızla kalsifikasyon gelişmekte, kalsifiye arterlerde de MGP-mRNA ekspresyonunun artışına neden olmaktadır. Bu nedenle warfarin kullanımı damar duvarında kalsifikasyon artışıyla sonuçlanabilir. Biz çalışmamızda warfarinin koroner arterlerde kalsifikasyona neden olup olmadığını araştırmayı amaçladık. Ortalama 66±46 ay önce protez kapak cerrahisi uygulanmış 39 hasta (ortalama yaşları 54±15 yıl) çalışmaya alındı. Kontrol grubu (n=28) herhangi bir tıbbi problemi olmayan yaş ortalamaları 52±11 yıl olanlardan seçildi. Tüm olgularda multidedektör bilgisayarlı tomografi ile kalsiyum skoru belirlendi. Çalışma ve kontrol gruplarında koroner kalsiyum skorunu birbirine benzer bulduk. Ek olarak hasta grubunda warfarin kullanım süresi koroner kalsiyum skoru ile korele değildi. Erkek cinsiyet, hipertansiyon ve LDL-kolesterol koroner kalsifikasyonun bağımsız belirteçleri olarak bulundu. Çalışmamızın sonuçları en azından orta yaşlı erişkinlerde warfarin kullanımının koroner kalsifikasyon üzerinde etkisinin olmadığını göstermektedir.

Anahtar kelimeler: *Koroner kalsifikasyon, multidetektör bilgisayarlı tomografi, warfarin*

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Date submitted: February 10, 2010 • **Date accepted:** November 12, 2010

Introduction

Warfarin is a widely used agent in cardiovascular practice, especially in patients with prosthetic valves. Some recent reports have revealed that warfarin inhibits the matrix γ-carboxyglutamic acid protein (MGP), which acts as a calcification inhibitor in vivo (1). In humans, defects in the MGP gene that predict a nonfunctional MGP protein have been shown to be responsible for Keutel syndrome (2). This inherited rare disease is characterized by abnormal calcification of cartilages, including costal, nasal, auricle, tracheal, and growth plate cartilage, nasal hypoplasia and brachtelephalangia; and by multiple peripheral pulmonary artery stenoses (3,4). In mice, targeted deletion of the MGP-gene causes rapid calcification of the elastic lamellae of the arterial media that begins at birth and is sufficiently extensive by 3 to 6 weeks of age that the arteries become rigid tubes that fracture, causing death by exsanguination in most of the affected mice by 6 weeks of age (5). Treatment of rats with the vitamin K antagonist, warfarin, at doses that inhibit the γ-carboxylation of MGP causes rapid calcification of elastic lamellae of arteries and of aortic heart valves and increased expression of MGP mRNA in the calcifying artery (1). Calcification is a common finding in the pathophysiology of the aging human artery and heart valve and is associated with several cardiovascular disease states. Warfarin-induced calcification in arteries is observed to occur only in the growing animal, and neither seen in older, nongrowing rats nor in young rats whose growth was temporarily arrested by a calorically-restricted diet. Besides more recent studies speculated that serum MGP levels were inversely correlated with the severity of of coronary artery calcium load (6), and oral anticoagulation might be associated with increased valvular and coronary calcium in patients with aortic valve disease (7).

Multidetector computed tomography was previously shown to be sensitive in determining coronary

artery calcification (8-10). Our aim was to investigate the effect of warfarin use on coronary calcification detected by multidetector computed tomography in patients with mechanical prosthetic heart valves due to rheumatic valve disease.

Material and Methods

In this cross-sectional observational study a total of 67 subjects were studied. Study group (Group I) was composed of 39 patients (11 men and 28 women) with a mean age of 54±15 years, who underwent prosthetic heart valve surgery 66±46 months ago due to rheumatic valve disease. All patients were under warfarin therapy at least for 36 months, with targeted INR value between 2.5-3.5, and no history of thromboembolism and bleeding attributed to warfarin was present. Twenty eight age- and gender-matched healthy subjects (9 male and 19 female) with a mean age of 53±12 years comprised the control group (Group II). All patients were provided informed consent before the investigation in accordance with local ethics committee requirements.

Prior to the study enrollment, blood lipid analysis was performed in all subjects. Patients with triglyceride levels higher than 400 mg/dl (due to the inability to calculate low-density lipoprotein values using Friedewald formula), patients with lipid-lowering medication and patients with parathyroid disease, increased serum calcium levels, and renal failure were excluded.

Hypertension was defined as the blood pressure over 140/90 mm Hg after 5 minutes rest or using antihypertensive medication, and diabetes mellitus was defined as fasting plasma glucose level above 126 mg/dl or using antidiabetic medication. Weight measurements were done with light clothes by using calibrated electronic scale, and height measurements with naked foot. Smokers were defined as current smokers or ex smokers.

Multidetector computed tomography (MDCT) was performed in every subject using 16-slice CT scanner (MX8000 IDT, Philiphs Medical Systems). To measure the calcium score, load and mass (prospective electrocardiogram [ECG]-gated made, the collimation space: 0.625 mm, rotation time 0.5 sec, scan time 250 msec, tube voltage 120 kV, pitch 0.275, tube current 165 mAS) with the workstation (Philiphs, Extended Brilliance TM, workspace, Release 1.0.1.). The calcium score were measured according the method of Agatston (11). On the basis of the Agatston score, the patients were separated into the categories proposed by Rumberger et al. (12): no calcification (calcium score 0), minimal calcification (calcium score 1-10), mild calcification (calcium score 11-100), moderate calcification (calcium score 101-400), or severe calcification (calcium score >400). Subjects with a heart rate over 70 were administered intravenous esmolol in order to slow down the heart rate and obtain clear images.

The outcome variable for this study was coronary calcium score. The primary predictor variable was the time period of warfarin therapy with covariates of age, LDL-C, HDL-C, total cholesterol, body mass index (BMI), diagnosis of hypertension, DM, current and past tobacco use.

In statistical analysis parametric values were expressed as mean±1 standard deviation, and nonparametric values as percentage. Mann Whitney U and Chi square for non parametric values, and independent samples t test for parametric values were used in order to compare the groups. Multiple linear regression analysis was used to evaluate the patient contributions of age, gender, serum lipids and presence of warfarin treatment for the amount of coronary calcium. A p value <0.05 was set significant.

Results

The average heart rates of the subjects were 68±7 and 66±6 for Group I, and Group II, respectively. The prosthetic heart valves were mitral, aortic, and mitral and aortic prosthesis in 20 (%52), 6 (15%), and 13 (33%) patients, respectively. Of these patients with mitral prosthesis 15 were female, and 5 were male. Aortic valve prosthesis was observed in 3 female and 3 male patients. All the patients with mitral and aortic prostheses were female.

The mean total calcium score of Group I and Group II were 27±55 and 21±60, respectively. In Group I, 19 patients (48.7%) did not have any detectable calcium, 12 (30.8%) had minimal calcification, 5 (12.8%) had mild calcification and 3 (7.6%) had moderate calcification at their coronary arteries. None of the cases had severe coronary calcification. On the other hand, no calcium was detected in 16 subjects, minimal calcification in 3 subjects, and moderate calcification in 3 subjects in Group II. Neither total calcium score difference between Group I and II, nor the risk classification of the coronary calcification was significantly different between the groups. The data and the statistical analysis of the groups are shown in Table I.

We found no correlation between the coronary calcification score and the duration of warfarin therapy (r=0.09). The subgroup analysis of the study group revealed that mean warfarin therapy period was 80±53 months in patients with no coronary calcification, and 44±35 months in patients with minimal calcification, and 50±36 months in patients with mild cal-

Table I. The data of the study group and control group and their statistical comparison

	Group I (Study group)	*Group II (Control)*	*p value*
Gender			0.730
Male	11 (28.2%)	9 (32.1)	
Female	28 (71.8%)	19 (67.9)	
Age (year)	54±15	53±12	0.238
Hypertension	5 (12.8%)	0	
Diabetes mellitus	4 (10.3%)	0	
Body mass index (kg/m^2)	25.54±3.56	24.71±3.77	0.728
Cholesterol (mg/dl)	186.66±42.39	192.79±33.07	0.380
Triglyceride (mg/dl)	129.14±46.74	134.62±42.05	0.588
HDL-C (mg/dl)	46.7±11.37	41.34±12.84	0.640
LDL-C (mg/dl)	112.35±38.34	118.03±34.24	0.944
Total coronary calcium score	26.63±54.60	20.90±59.70	0.408
Risk classification of coronary calcification	19 (48.7%) no calcification 12 (30.8%) minimal calcification 5 (12.8%) mild calcification 3 (7.6%) moderate calcification	16 (57.1%) no calcification 6 (21.4%) minimal calcification 3 (10.7%) mild calcification 3 (10.7%) moderate calcification	0.666

cification and 103±10 months in patients with moderate calcification, and the difference between the subgroups was not significant (χ^2=51.297; p=0.462). Multiple linear regression analysis revealed that there was no relationship between the length of warfarin use and calcification score (p=0.299). There were also no correlations between coronary calcification and the BMI (r=0.01; p=0.647), triglyceride levels (r=0.04; p=0.343), and age (r=0.08; p=0.217), whereas the correlation coefficients between coronary calcification and gender, total cholesterol, LDL-cholesterol and HDL-cholesterol were r=0.30 (p=0.01), r=0.26 (p=0.022), r=0.23 (p=0.02) and r=-0.22 (p=0.018), respectively. Multiple linear regression analysis revealed that male gender (p=0.031), arterial hypertension (p=0.04), and LDL-C (p=0.022) were independent predictors of the coronary calcification.

Discussion

This study has shown that long-term warfarin use is not associated with excessive coronary calcification. Coronary calcification has been accepted as a sensitive and specific marker for atherosclerosis. Calcification of the intimal layer of the artery is observed in the setting of atherosclerotic plaque (13,14). Several models have been proposed to explain the development of intimal calcification: a model of an active process in which there is formation of bone like materials (15,16), a physicochemical model, and an arterial osteoclast-like cell model (17). According to the physicochemical model, there are inhibitors in arteries that prevent precipitation of calcium, at least in part by chelating calcium ions (18-22). Several proteins that might modulate calcium precipitation in the extracelluler fluid contain γ-carboxyglutamic acid (Gla) amino acid residues, which can chelate or bind calcium ions. Gla-containing proteins also include bone proteins such as matrix Gla protein (MGP) (23-27). Numerous studies are consistent with the idea that MGP prevents precipitation of calcium mineral in arteries (1, 28-31).

The activity of MGP is dependent on carboxylation using Vit K as a cofactor. Although in an animal model, low intake of Vit K has been shown to accelerate vascular calcification via decreased MGP activity, it has been found that dietary Vit K1 (phylloquinone) intake was not correlated with coronary calcification (32). The hypothesis that, warfarin, Vit-K antagonist, inhibits the MGP activity and cause vascular calcification has been tested in animal models and rapid calcification of elastic lamellae and of aortic heart valves have been observed (1,31). Although some case reports have claimed that warfarin therapy is associated with coronary and tracheobronchial calcification (33,34), results of our study reveal that warfarin treatment is not associated with coronary calcification in adult humans. Strikingly, children under warfarin therapy are susceptible for tracheobronchial calcification (35). Similarly in an animal study (31), warfarin treatment caused massive focal calcification of the artery media in 20-day-old rats and less extensive focal calcification in 42-day-old rats, and finally no calcification was found in 10-month-old adult rats. Price et al. have concluded that warfarin-induced calcification may be promoted by increase in serum calcium or phosphate, which are found in higher serum levels in younger rats or by metabolic processes that are activated by growth and by vitamin D (31). This

finding may explain the absence of relation between warfarin use and coronary calcification. All of our patients were adult, whose bone development were completed. Besides, the patients with disease that might increase serum calcium level were excluded.

Another explanation might be that arterial calcifying effect of warfarin may be dose dependent. The doses used in animal studies are higher than the therapeutic dose for humans, for example, Price et al. administered 15 mg warfarin/100 g body weight/day to rats (31). In our study, maximum daily dose was 0.028 mg/100 g body weight. This is very low compared to the study of Price et al.

The lack of correlation might be also explained by the different structure of the coronary arteries. In experimental models, warfarin-induced vascular calcification is attributed to precipitation of calcium at elastic lamellae of the media of artery (1,31). Because human coronary arteries have thin media and elastic lamellae, the calcifying effect might have been so small to be detected by tomography.

Previous studies indicated a relationship between hyperlipidemia and vascular, and also cardiac valve calcification independent of age (36-39). Our study also showed that coronary calcification is positively correlated with gender, total cholesterol, LDL-cholesterol and negatively correlated with HDL-cholesterol, which is similar to those papers previously reported. There was no correlation found between coronary calcification and BMI, triglyceride levels, and age in our study. These results are coherent to that of those papers.

A very recent study done by multidetector computerized tomography found a relationship between warfarin and aortic valvular, and coronary calcification (7). In that study while treatment of warfarin was only independent predictor for aortic valvuler calcification, male gender, hypertension, and warfarin treatment were independent predictors for coronary calcification. In our study we evaluated only coronary calcification, and determined that male gender, hypertension, and LDL-C but not warfarin treatment were independent predictors for coronary calcification. The discrepancy between two studies may be explained by our younger patients and shorter duration of warfarin treatment. However, more important difference was the patient population of our study, which was composed of those with all heart valve replacement due to rheumatic heart disease. The study group of the paper published by Koos et al. included those with old aortic valve stenosis, probably due to aortic calcification (7). Wang et al. declared that gene polymorphism of vitamin K epoxide reductase subunit 1 is associated with stroke, coronary artery disease, and aortic dissection (40). The risk of calcification induced by warfarin may be associated with this pronounced gene polimorphism. Readily there are no data about this idea. We specially chose the patients with heart-valve prosthesis because warfarin is widely used in this population and coronary calcification associated with warfarin use is an important concern in these patients. Our study revealed that long term usage of warfarin is safe with regard to coronary calcification.

In conclusion the data of our study imply that warfarin use is not associated with increased coronary calcification when compared to the control group. The lack of difference could be attributed to susceptibility to calcification. However, the lack of relationship between the duration of warfarin use and coronary calcification score also supports the conclusion that warfarin use in therapeutic doses is not associated with coronary calcification, at least in adult humans.

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