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Besirli, Kazim; Aydemir, Birsen; Arslan, Caner; Riza Kiziler, Ali; Canturk, Emir; Kayhan, Bekir

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Calcium dobesilate may improve hemorheology in patients undergoing coronary artery bypass grafting

Dobesilato de cálcio pode melhorar hemorreologia em pacientes submetidos à cirurgia de revascularização miocárdica

Kazim Besirli[1], Birsen Aydemir[2], Caner Arslan[1], Ali Riza Kiziler[3], Emir Canturk[4], Bekir Kayhan[4]

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Abstract

Background: **Calcium dobesilate is an angioprotective agent that has positive effects on hemorheological parameters. It is an antioxidant that increases endothelial-derived vasodilator substance secretion, there are none that analyze its effects during the postoperative period of patients undergoing myocardial revascularization.**

Objective: **We aimed to determine the effects of calcium dobesilate on hemorheological parameters, such as reduced glutathione and malondialdehyde in patients with ischemic heart disease undergoing myocardial revascularization in the postoperative period.**

Methods: **One hundred and thirty-four patients operated for coronary heart disease were included in this study. Hemorheological, oxidant and antioxidant parameters were measured two days after surgery and after a period of treatment with calcium dobesilate. Then, 500 mg of calcium dobesilate was given twice a day to one group of 68 patients for three months. The control group was composed of 66 patients who did not receive this medication.**

Results: **The increase in the erythrocyte deformability index was found to be significant compared with both the pretreatment values and with the 1st and 2nd values of the control group after calcium dobesilate administration, whereas there were no significant changes in blood viscosity, glutathione (GSH) or malondialdehyde (MDA) values after the calcium dobesilate administration. The same improvement in the CCS class was observed in patients regardless of they received the calcium dobesilate treatment.**

Conclusion: **In the present investigation, the same improvement in the CCS class was observed in patients regardless of they received the calcium dobesilate treatment. Improvements with calcium dobesilate were statistically significant only in the increase in erythrocyte flexibility.**

Descriptors: **Coronary artery disease. Atherosclerosis. Coronary artery bypass.**

Resumo

Antecedentes: **O dobesilato de cálcio é um agente angioprotetor que tem efeitos positivos sobre os parâmetros hemorreológicos. É um antioxidante que aumenta a secreção endotelial derivada da substância vasodilatadora, não há nada que analisar os seus efeitos durante o período pós-operatório de pacientes submetidos a revascularização do miocárdio.**

Objetivo: **Nosso objetivo foi determinar os efeitos de dobesilato de cálcio sobre os parâmetros hemorreológicos, tais como glutationa reduzida e malondialdeído em pacientes com doença cardíaca isquêmica submetidos a revascularização do miocárdio no pós-operatório.**

1. Istanbul University, Cerrahpasa Medical Faculty, Cardiovascular Surgery Department, Istanbul, Turkey.
2. Istanbul University Cerrahpasa Medical Faculty, Department of Biophysics, Istanbul, Turkey.
3. Namik Kemal University, Medical Faculty, Biophysics Department, Tekirdag, Turkey.
4. TDV 29 Mayis Hospital, Cardiovascular Surgery Department, Istanbul, Turkey.

Correspondence address
Birsen Aydemir
Istanbul University - Department of Biophysics Fatih - Cerrahpasa Medical Faculty – 34098. Istanbul, Turkey
E-mail: birsenay2001@yahoo.com

Work performed at Istanbul University Cerrahpasa Medical Faculty Department of Biophysics, Istanbul, Turkey.

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Abbreviations, acronyms & symbols	
BHT	Butylhydroxytoluene
BV	Blood viscosity
BV	Blood viscosity
CCS	Canadian Cardiac Society
CCS	Canadian Cardiac Class
DTNB	Nitrobenzoic acid
EDI	Erythrocyte deformability index
EDTA	Ethylenediaminetetraacetate
EF	Ejection fraction
GSH	Glutathione
Hct	Hematocrit
MDA	Malondialdehyde
PV	Plasma viscosity
ROS	Reactive oxygen species
TBARS	Thiobarbituric acid-reactive substances
TCA	Trichloroacetic acid

Métodos: **Cento e trinta e quatro pacientes operados por doença cardíaca coronária foram incluídos neste estudo. Parâmetros de oxidante, hemorreológicos e de antioxidantes foram medidos dois dias após a cirurgia e após um período de tratamento com o dobesilato de cálcio. Em seguida, 500 mg de dobesilato de cálcio foi administrado duas vezes por dia para um grupo de 68 pacientes durante três meses. O grupo controle foi composto por 66 pacientes que não receberam essa medicação.**

Resultados: **O aumento do índice de deformabilidade dos eritrócitos foi considerado significativo comparado com ambos os valores pré-tratamento e com os 1º e 2º valores do grupo controle após a administração dobesilato de cálcio, enquanto que não houve alterações significativas na viscosidade do sangue, na glutationa (GSH) ou malondialdeído (MDA) após a administração dobesilato de cálcio. A mesma melhoria na classe CCS foi observada em pacientes independentemente de terem recebido tratamento com dobesilato de cálcio.**

Conclusão: **Na presente investigação, a mesma melhora na classe CCS foi observada em pacientes independentemente de terem recebido o tratamento com dobesilato de cálcio.**

Descritores: **Doença da artéria coronariana. Aterosclerose. Ponte de artéria coronária.**

INTRODUCTION

Myocardial ischemia and infarction are the ultimate results of coronary artery disease . Hemorheological factors that can worsen tissue's ischemia may accentuate the disease. Fibrinogen has a pronounced effect on plasma viscosity (PV). Hematocrit (Hct) and PV are the most important components of blood viscosity (BV) and PV also plays an important role in the atherosclerotic process [1,2]. Atherogenesis is further accelerated by an impaired blood flow that is closely related BV [3].

It has been shown that drugs that may increase erythrocyte flexibility decreased pain in critical limb ischemia during rest [4]. Reactive oxygen species (ROS) may result in cell injury and cause oxidative damage to lipids which is an important component of atherosclerotic cardiovascular heart disease. Erythrocytes reduce glutathione (GSH), which is an endogenous mechanism of oxidant inactivation; accordingly, once formed, ROS oxidizes GSH which is then released outside the cells and prevents oxidative damage [5].

Calcium dobesilate which is a veno-tonic drug, has long been used effectively in many countries for the treatment of diabetic retinopathy, chronic venous insufficiency and symptoms of hemorrhoidal attacks. In recent years, it has also been shown that calcium dobesilate can improve hemorheology and microcirculation and possesses antioxidant, and antiplatelet properties, as described for its clinical and experimental use [1,6]. However, there are no reports analyzing the effects of calcium dobesilate in the postoperative period of patients undergoing myocardial revascularization. This study aimed, therefore, to investigate whether administration of calcium dobesilate would exhibit any beneficial effects on hemorheology and oxidative stress in the postoperative period of patients undergoing myocardial revascularization.

METHODS

A total of 134 subjects (110 men and 24 women) who underwent coronary bypass grafting were included in the present study. Patient selection was non-randomized. Sixty-eight subjects (58 men and 10 women, mean age: 54.6 ± 6.1 years) received 500 mg calcium dobesilate twice a day following the first blood samples that were taken 2 days after surgery. Sixty-six subjects (52 men and 14 women, mean age: 55.4 ± 5.2 years) who did not take this medication comprised the control group. All of the coronary bypass operations were performed on pump in the study group. Preoperatively, in the group receiving calcium dobesilate, 32 patients had CCS 4 status and 36 patients had CCS 2-3 status, whereas in the control group, 30 patients had CCS 4 status and 36 patients had CCS 2-3 status. Regarding cardiovascular risk factors for all patients, 98 patients were ex-smokers (they had stopped smoking just before the operation), 50 had a family history of cardiovascular disease, hypercholesterolemia was present in 71, diabetes mellitus was present in 29 and essential hypertension was present in 63. Twenty-five patients had been taking statins preoperatively. The left ventricular ejection fraction in 70

patients was >50%, 35%-50% in 44 patients and <35% in 20 patients. Four-vessel bypasses had been performed in 18 patients, three-vessel bypasses had been performed in 34, two-vessel bypasses had been performed in 66 and a one-vessel bypass had been performed in 16 patients (Table 1). This study conforms with the Helsinki Declaration of the World Medical Association, and the Ethics Committee of the Cerrahpaºa Medical Faculty approved the research protocol. Informed consent was obtained from each patient after receiving verbal and written information about the study.

Three months after the surgery, 10 patients in the calcium dobesilate group had CCS 1-2 status, whereas eight patients in the control group had CCS 1-2 status. Pleural effusions were observed in two patients in the calcium dobesilate group and in three patients in the control group. Postoperative atrial fibrillations developed in five patients in the calcium dobesilate group and in four patients in the control group. No early or late mortality was observed in either group.

Blood samples were drawn from the patients' antecubital veins after 12-hour fasting. Initial blood samples were taken two days after the operation, and the second samples were taken 3 months later. In this period, 68 patients had taken calcium dobesilate 500 mg twice a day and a placebo was given to the 66 patients in the control group.

Routine blood counts (for Hct) were determined with ethylenediaminetetraacetate (EDTA)-anticoagulated blood samples by an electronic counter (Medonic CA 570, Sweden).

To determine the erythrocyte deformability index (EDI), plasma and blood viscosity, erythrocyte and plasma malondialdehyde (MDA) and GSH, blood samples were collected in vacutainer tubes containing EDTA without anticoagulant. Fibrinogen was collected in vacutainer tubes containing sodium oxalate. Plasma samples were obtained by centrifugation at 1000 x g for 20 min and stored at –70°C. The erythrocytes were prepared with whole blood centrifugation for 5 min at 1000 x g and obtained after washing in a 0.9% NaCl solution twice and then removed for measurement.

Lipid peroxide levels were measured in the plasma and erythrocytes using a thiobarbituric acid-reactive substances (TBARS) assay, which monitors MDA production [7]. Briefly, to a 200 µl sample containing erythrocyte pellet and plasma, 800 µl phosphate buffer (pH 7.4), 25 µl butylhydroxytoluene (BHT) (88 mg/10 ml absolute alcohol) and 500 µl of 30% trichloroacetic acid (TCA) was added and mixed. After 2 h incubation at 20 °C, the mixture was centrifuged (400 g) for 15 min. After this, 1 ml supernatant was added to each tube, followed by the addition of 75 µl of 0.1 M EDTA and 250 µl of 1% thiobarbituric acid (TBA). The tubes, which had teflon-lined screw caps, were incubated at 90°C in a water bath for 15 min and cooled to room temperature. The optical density was measured at 532 and 600 nm by ultraviolet-visible spectrophotometry for the erythrocyte MDA and at 532 nm for the plasma MDA and tissue MDA concentrations (Shimadzu UV-1601, Tokyo, Japan). The MDA level was determined using the molar absorption coefficient of the MDA, $1.56x10^5\ M^{-1}cm^{-1}$ at 532 nm.

The GSH concentration was determined in the erythrocytes by a modified coupled optical test system [8]. In this system, GSH is oxidized by 5,5'dithiobis-2 nitrobenzoic acid (DTNB) and then reduced by GSH reductase with NADPH as the hydrogen donor. The oxidation of GSH by DTNB was detected photometrically by a change in the absorption at 412 nm. Briefly, to a 100 µl

Table 1. Characteristics of patients in calcium dobesilate group and control group.

	Calcium Dobesilate Group	Control Group	*P*
Number (Male/Female)	68 (58/10)	66 (52/14)	0.326
Age Mean	54.6 ± 6.1	55.4 ± 5.2	0.761
Preop CCS Class	Class 4; 32, Class 2-3; 36	Class 4; 30, Class 2-3; 36	0.852
Postop CCS Class (3 months after operation)	Class 1-2; 10	Class 1-2; 8	0.661
Pleural effusion	3	2	0.673
Atrial fibrillation	5	4	0.765
Number of bypasses (4/3/2/1)	8/16/32/12	10/18/34/4	0.224
EF (>50%/50%-35%/<35%)	36/20/12 patients	34/24/8 patients	0.551
Ex-smoker	52	46	0.376
Family history	30	20	0.098
Hypercholestrolemia	41	30	0.085
Diabetes mellitus	14	15	0.764
Essential hypertension	37	26	0.082
Preop statin use	12	13	0.761

EF = Ejection Fraction; CCS = Canadian Cardiac Society

sample, 150 µl of 5% sulfosalicylic acid (w/v) was added to induce lysis. Twenty µl of lysate was added to 980 µl of reaction buffer (100 nM potassium phosphate buffer, 1 mM NADPH, 0.5 mM DTNB, 0.5 U GSH reductase pH 7.4). The change in absorption was recorded at 412 nm with ultraviolet-visible spectrophotometry (Shimadzu UV-1601, Tokyo, Japan). The GSH level was determined using the molar absorption coefficient of GSH at 412 nm $13.6x10^{-4}$ $M^{-1}cm^{-1}$.

The BV and the PV were measured using a Harkness Capillary Viscometer (Coulter Electronics Ltd, Ser. No: 6083, England) at 37 (relative viscosity) [9]. Erythrocyte deformability was determined by a stroboscopic centrifugal method. Plasma fibrinogen levels were measured using calorimetric kits (Sigma Chemical and Fibri-Prest, Diagnostica Stago, France).

Statistical analysis

All results are expressed as the mean and standard deviation (SD). The statistical significance of differences was determined by SPSS version 15.0 for Windows (SPSS, Chicago, IL, USA). Patient characteristics were compared using Pearson Chi square test for categorical data. The Student's t-test, Mann-Whitney U test, the one–way analysis of variance and Tukey's honestly different significance test were used to evaluate the significance of differences in the parameters of age, whole blood viscosity, plasma viscosity, the erythrocyte deformability index, erythrocyte reduced glutathione levels, erythrocyte malondialdehyde levels, plasma malondialdehyde levels, fibrinogen levels and Hct among both the pretreatment and posttreatment values in the calcium dobesilate group and the first and second values in the control group. $P< 0.05$ was considered statistically significant.

RESULTS

The mean values of BV, PV, Hct, EDI, plasma fibrinogen, erythrocyte GSH, erythrocyte MDA and plasma MDA are shown in Table 2 and 3.

The EDI values after 3 months of medication with calcium dobesilate were found to be statistically significantly higher than both those before treatment ($P<0.001$) and the first and second values in the control group ($P<0.01$ and $P<0.01$, respectively).

The decrease in BV, PV, Hct and erythrocyte GSH and the increase in plasma fibrinogen, erythrocyte MDA and plasma MDA values were not statistically significant.

Table 2. BV, PV, Hct and EDI values before and after calcium dobesilate treatment in the calcium dobesilate group and first and second values after 3 months in the control group.

Parameters	Calcium Dobesilate Group (Pretreatment values)	Calcium Dobesilate Group (Postreatment values)	[a]*P*	Control Group (First values)	[b]*P*	Control Group (Second values)	[c]*P*	[d]*P*	[e]*P*	[f]*P*
Whole blood viscosity (m.Pas)	3.38±0.44	3.14±0.44	0.105	3.24±0.12	0.731	3.13±0.15	0.998	0.463	0.065	0.656
Plasma viscosity (m.Pas)	1.39±0.25	1.38±0.38	0.996	1.35±0.12	0.982	1.38±0.09	0.998	0.926	0.998	0.955
Hct (%)	33.22±1.24	32.76±1.44	0.987	33.67±1.74	0.453	32.42±1.59	0.804	0.677	0.590	0.059
Erythrocyte deformability index (%Hct min-1)	9.82±0.94	11.13±0.76	0.001	10.16±1.00	0.01	10.19±0.77	0.01	0.596	0.502	0.998

The values are given as mean± SD.

[a]P = pretreatment values in calcium dobesilate group compared with posttreatment values in calcium dobesilate group (ANOVA, Tukey's Range [HSD] test); [b]P = first values in the control group compared with posttreatment values in the calcium dobesilate group; [c]P = second values in the control group compared with posttreatment values in the calcium dobesilate group; [d]P = first values in the control groupcompared with pretreatment values in the calcium dobesilate group; [e]P = second values in the control group compared with pretreatment values in the calcium dobesilate group; [f]P = first values in the control group compared with second values in the control group

Table 3. Fibrinogen, erythrocyte GSH, erythrocyte MDA and plasma MDA values before and after calcium dobesilate treatment in the calcium dobesilate group and first and second values after three months in the control group.

Parameters	Calcium Dobesilate Group (Pretreatment values)	Calcium Dobesilate Group (Postreatment values)	[a]*P*	Control Group (First values)	[b]*P*	Control Group (Second values)	[c]*P*	[d]*P*	[e]*P*	[f]*P*
Fibrinogen (mg/dL)	276.76±75.21	278.82±84.65	0.998	265.50±24.34	0.875	246.25±32.67	0.263	0.920	0.319	0.632
Erythrocyte GSH (mmol/g Hb)	15.59±2.03	14.30±2.39	0.265	14.74±2.24	0.936	14.57±2.79	0.935	0.496	0.504	0.998
Erythrocyte MDA (nmol MDA/g Hb)	3.05±0.63	3.15±0.54	0.975	3.09±0.40	0.964	3.23±0.48	0.956	0.998	0.767	0.697
Plasma MDA (nmol/mL)	5.88±1.05	6.04±1.31	0.946	5.92±0.55	0.969	6.02±0.39	0.998	0.998	0.956	0.977

The values are given as mean± SD.

[a]P = *pretreatment values in calcium dobesilate group compared with post treatment values in calcium dobesilate group (ANOVA, Tukey's Range [HSD] test);* [b]P = *first values in the control group compared with posttreatment values in the calcium dobesilate group;* [c]P = *second values in the control group compared with post treatment values in the calcium dobesilate group;* [d]P = *first values in the control group compared with pretreatment values in the calcium dobesilate group;* [e]P = *second values in the control group compared with pretreatment values in the calcium dobesilate group;* [f]P = *first values in the control group compared with second values in the control group*

DISCUSSION

In advanced atherosclerotic disease, the fluidity of the bloodstream is decreased. The classic hemorheological parameters, BV and PV, are higher than in healthy persons. In atherosclerotic disease, with its critically decreased pressure gradients and exhausted vascular reserves, an increased structural viscosity leads to reduced organ perfusion [10]. Approximately 40% of all vascular events can be explained by the classical risk factors for atherosclerotic disease [11]. In the early stages of atherosclerotic vascular disease, endothelium-derived relaxing factor release is diminished, and, in the later stages, plaques or stenosis cause vascular diameter regulation to deteriorate [12]. In patients for whom these problems cannot be modified, only hemorheological therapeutic interventions remain [12]. Rheological parameters, such as fibrinogen concentration, plasma viscosity and leukocyte count, are important risk factors for ischemic heart disease [12-14]. Oxidation and the production of free radicals are an integral part of the human metabolism [15]. Lipid oxidation is a significant, harmful consequence of ROS formation, as it reflects irreversible oxidative changes in membranes [16-19]. Plasma MDA levels are one of the most commonly used markers of lipid peroxidation. Increased venous concentration of MDA has been found in patients subjected to cardiac surgery [20-22]. However, an MDA concentration in systemic blood may reflect changes unrelated to the cardiac oxidative stress (prostanoid synthesis) activity of aldehyde-dehydrogenase and aldose reductase [23-25]. Despite calcium dobesilate therapy, an increase in erythrocyte and plasma MDA levels and a decrease in erythrocyte GSH in the present study shows that calcium dobesilate is not an effective antioxidant, which is in contrast to the studies that have demonstrated its antioxidant properties [26].

It has been shown that calcium dobesilate improves hemorheology by reducing BV, PV and Hct and potentiating fibrinolysis in diabetic patients [27]. The fact that calcium dobesilate decreases vascular permeability indicates that it plays a role in causing fluid retention in the vascular system, causing hemodilution [28]. An earlier and more rapid improvement was observed in patients with myocardial infarction who were taking CLS 2210 (a new formulation of calcium dobesilate) [29]. In an experimental study, it was shown that the same drug reduced mortality in rats after the occlusion of a coronary artery [30].

In this study, the decrease in the BV and PV and the increase in the plasma fibrinogen level in patients were not significant after 3 months of doxium medication. This increase was likely due to the increased hepatic production of fibrinogen related to postoperative stress [31]. The most beneficial effect of the calcium dobesilate treatment in our patient group was the significant increase in EDI when compared with the control group. Although the decrease in BV and PV values were not statistically significant after calcium dobesilate treatment in our study, a decreased BV is beneficial for coronary heart disease patients because a high BV could cause thrombosis by decreasing the dilution of activated coagulation factors and retarding the inflow of clotting inhibitors [3].

Elevations in PV and BV may aggravate hypoxia by increasing the resistance to flow in patients with peripheral arterial occlusive disease [32]. Deformability enables erythrocytes to pass through the nutritive capillaries, which have a diameter approximately half of theirs, and to supply oxygen to tissues. Erythrocytes lose these functions and become more susceptible to hemolysis if their flexibility falls below a threshold level [33]. In the present study, the only statistically significant result showing improvement because of calcium dobesilate administration was the increase in the flexibility of the RBC. It may also somewhat

improve coronary microcirculation when used as an adjunctive treatment in patients with ischemic heart disease, but it is illogical to relate the improvements in the CCS class to this drug in the calcium dobesilate group, because the same improvements were also observed in the control group, and all the patients were revascularized as much as possible.

Limitations

There are some limitations to our study. It was not blinded or randomized. We were also unable to administer calcium dobesilate to patients preoperatively because of the hospitalization policy. We were also unable to discontinue patients' anti-anginal and antiplatelet drugs while administering calcium dobesilate and could therefore not prevent interactions between these drugs.

CONCLUSION

In the present investigation, the same improvements in the CCS class were observed in patients regardless of whether they received calcium dobesilate. Calcium dobesilate was effective only for the increase in red blood cell flexibility.

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