

Protective effects of vitamin C on endothelium damage and platelet activation during myocardial infarction in patients with sustained generation of circulating microparticles

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Summary. During myocardial infarction (MI), high levels of circulating procoagulant microparticles (MP) shed from endothelial cells and platelets diffuse prothrombotic and proinflammatory potentials crucial for the coronary prognosis. In addition to conventional treatments, we evaluated whether vitamin C treatment could modify circulating levels of procoagulant MP. Upon admission, 61 patients with MI were prospectively randomized for immediate additional vitamin C treatment. Circulating MP were quantified by functional prothrombinase assay before and after 5 days of vitamin C administration (1 g day⁻¹). The cellular origin of MP was also assessed. In vitamin C-treated patients, the reduction in platelet-derived MP was 10% higher ($P = 0.01$). In patients with diabetes mellitus, dyslipidemia or more than two cardiovascular risk factors, vitamin C decreased endothelial and platelet-derived MP levels by ~70% and 13%, respectively. This early effect on circulating platelet and endothelial-derived MP, testifies to the importance of oxidative stress during MI. Vitamin C could prove beneficial for the outcome of patients at higher thrombotic risk.

Keywords: atherosclerosis; oxidative stress; thrombosis.

Introduction

Membrane microparticles (MP) are shed from stimulated and/or apoptotic vascular cells and released in blood flow. They bear membrane glycoproteins testifying to their cell origin and their amount was found to be correlated to the degree of apoptosis [1]. Elevated levels of circulating MP have been reported in a variety of pathologies [1–4]. In the blood flow, circulating MP provide an additional procoagulant phospholipid surface necessary for the assembly of the clotting enzymes complexes and

thrombin generation [5]. Their procoagulant potential relies on phosphatidylserine (PtdSer), an aminophospholipid translocated to the external membrane leaflet, and on the possible presence of membrane tissue factor (TF), the major cellular activator, expressed by smooth muscle cells, fibroblasts, monocytes and activated endothelial cells. High amounts of circulating procoagulant MP, shed mainly from platelets and endothelial cells, are detected during acute coronary syndrome [6] and may influence the coronary prognosis [7].

Oxidative stress may account for a significant proportion in endothelium dysfunction and platelet activation observed in atherogenesis [8–12]. Evidence suggests that the antioxidant status is linked to the clinical expression of coronary artery disease [13]. Indeed, low vitamin C serum concentrations are associated with inflammation and severity of the illness [14,15]. Therefore, additional antioxidant treatments were proposed to reduce both platelet activation and endothelial dysfunction during atherogenesis [16]. In various experimental models, antioxidants were found to be beneficial on vasospasm, neointimal thickening or remodeling after balloon injury [17]. Accordingly, antioxidant vitamin C treatment was shown to improve endothelial function in several subsets of patients including hypercholesterolemia, coronary artery disease and heart transplantation vasculopathy [18–22].

In this study, we examined whether additional treatment by vitamin C may reduce circulating procoagulant MP as a marker of vascular damage and platelet activation in the peripheral blood of patients with myocardial infarction (MI). The cell origin of MP was also assessed to detect a possible subset of cells more responsive to vitamin C treatment. MP were isolated from plasma samples by capture onto immobilized annexin V, a protein with high affinity for PtdSer. MP measurements and characterization were performed using a modified prothrombinase assay [4]. Effects of vitamin C were examined with respect to various cardiovascular risk factors.

Patients and methods

Sixty-one patients presenting an acute myocardial infarction were enrolled in the study. Typical chest pain, persistent

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ST-segment elevation on electrocardiogram, and a two-fold rise in creatine phosphokinase (CPK) diagnosed MI. All patients received conventional treatment including beta-blockers, aspirin (160–325 mg day⁻¹) before blood sampling. An additional weight-adjusted unfractionated heparin regimen was applied (initial bolus 50 units kg⁻¹) to achieve an activated partial thromboplastin time between 60 and 90 s. Patients were prospectively randomized to receive an additional 5 days' vitamin C oral treatment (1 g day⁻¹) or placebo tablets (29 and 32 patients, respectively). 76% of the patients treated by vitamin C and 75% of the patients with placebo were submitted to primary angioplasty. Statins, anti-ischemic medications and anti-platelet inhibitors were equally prescribed. The opportunity of angioplasty, stent placement and additional anti-GPIIb/IIIa therapy (abciximab or eptifibatid) was under the responsibility of independent angioplasty physicians. Vitamin C was given just at the end of anti-GPIIb/IIIa treatment. This treatment consisted of one bolus of 250 µg kg⁻¹ followed by 0.125 µg kg⁻¹ min⁻¹ continuous infusion up to 18 h for abciximab and one bolus of 180 µg kg⁻¹ followed by initiation of 2 µg kg⁻¹ min⁻¹ continuous infusion up to 18 h for eptifibatid. Patients with diabetes mellitus (DM) were recruited on the basis of documented medical reports, if treated by insulin or oral hypoglycaemic agents, or when elevated levels of fasting blood glucose (> 140 mg dL⁻¹) were measured on at least two separate occasions. Dyslipidemic patients (DL) were identified on the basis of medical history, ongoing treatment by statins or fibrates, or high levels of total or LDL cholesterol measured during their stay. Other cardiovascular risk factors considered were arterial hypertension, current smoking, increase weight (body mass index >29 kg m⁻²). For comparative purpose, 23 patients with less than two cardiovascular risk factors were defined as low-risk patients (referred to as LR in the text) regardless of the occurrence of DM or DL. Thirty-eight patients with more than two cardiovascular risk factors, including DM and DL, were defined as high-risk patients (referred to as HR in the text). Fifty healthy volunteers (HV) were simultaneously investigated during the inclusion period as a reference group. Written informed consent was obtained from all the patients with the approval of local Ethical Committee (Comité Consultatif de Protection des Personnes dans la Recherche Biomédicale, Strasbourg).

Reagents

Monoclonal antibody (mAb) to human platelet glycoprotein GPIb was a kind gift of Dr F. Lanza, biotinylated as described elsewhere [4]. Biotinylated mAb to CD31 was from CALTAG Laboratories (Burlingame, CA, USA), and the irrelevant corresponding biotinylated immunoglobulins were from Leinco Technologies (Ballwin, MO, USA). Human prothrombin (FII) was from Hyphen BioMed (Andresy, France) and activated factor X (FXa) from Biogenic S.A. (Mauguio, France). Activated factor V (FVa) was a product from American Diagnostica (Greenwich, UK). Biotinylated recombinant human annexin V was the same as that used elsewhere [1]. High binding capacity

streptavidin-coated microtitration plates and Chromozym TH were from Roche Diagnostics (Mannheim, Germany).

Isolation of circulating MP and determination of their procoagulant potential

Blood samples, collected by venous puncture, were collected on 138 mmol L⁻¹ citrated solution (9 volumes of blood : 1 volume of anticoagulant) before vitamin C therapy [day (D)1] and 5 days later (D5). Platelet-free plasma samples (PFP) containing circulating MP were obtained by double centrifugation as previously described [1]. MP were captured onto insolubilized annexin V and their PhtdSer content was determined by functional prothrombinase assay using a microplate reader equipped with kinetics software. In this assay, blood clotting factors (FXa, FVa, FII) and calcium concentrations were determined to ensure that PhtdSer is the rate-limiting parameter in the generation of soluble thrombin from prothrombin. In this purified system, the presence of TF on captured MP does not alter values corresponding to PhtdSer content, and FVa was in excess with respect to FXa in order to exclude any contribution of FVa, possibly associated with MP. Results were expressed as nanomolar PhtdSer equivalent (nmol L⁻¹ PhtdSer Eq) by reference to a standard curve constructed with liposomes of known composition and concentration [1].

Search for the cellular origin of circulating MP

Biotinylated monoclonal antibodies to various cell types (anti-CD31 for endothelial cells, anti-GPIb for platelets) were insolubilized onto streptavidin-coated microtitration plates as previously described [4]. CD31 being also expressed to a small extent on platelets, it was previously ensured that circulating MP bearing CD31 ([CD31]⁺ MP) mainly originate from apoptotic endothelial cells and are therefore a reliable marker of endothelial damage [7,23]. After incubation of PFP and washing, captured MP were quantified by prothrombinase assay as described above. Background values obtained with corresponding irrelevant IgGs were subtracted. It should be emphasized that no direct comparison between capture by annexin V and antibodies could be performed because affinities for the respective counterpart ligands are different.

Statistical analysis

Results are expressed as mean ± SEM from at least two independent measurements. Patients groups were compared using a Mann–Whitney test. A *P*-value <0.05 was considered statistically significant.

Results

Patients

Clinical details, angiographic data and treatments are given in Tables 1 and 2. Sex, risk factor distribution, median time to therapy, localization of MI, multivessel disease, CPK peak, type

Table 1 Baseline characteristics and medical history

	Placebo (n = 32)	Vitamin C (n = 29)
Age (years)	62 ± 8	61 ± 9
Male/female	29/3	24/5
Current smoker (%)	76	58
Dyslipidemia (%)	56	62
Diabetes mellitus (%)	32	41
Arterial hypertension (%)	50	69
Time from onset of pain to intervention (h)	4.0	3.5
Peak creatine kinase (U L ⁻¹)	1395 ± 872	1745 ± 934
Left ventricular ejection fraction assessed at day 5 by echocardiography	0.51 ± 0.06	0.47 ± 0.06

Table 2 Angiographic data and treatments

	Placebo (n = 32)	Vitamin C (n = 29)
Primary angioplasty (%)	75	76
Multivessel disease (%)	55	86
Target vessel (%)		
Left anterior descending coronary	41	42
Left circumflex	3	16
Right coronary artery	55	42
TIMI 0/1 flow (%)	73	83
Stent placement (%)	83	59
Clopidogrel (%)	57	50
Ticlopidine (%)	26	20
ASA (%)	87	92
GPIIb/IIIa antagonist (%)	35	51
Heparin (%)	97	97

Coronary perfusion was assessed by TIMI flow scoring as defined by the Thrombolysis in myocardial infarction study. TIMI 0 is defined by the absence of antegrade flow, TIMI 1 by penetration of contrast with uncompleted opacification of the coronary vascular bed. ASA, acetylsalicylic acid.

of therapy were not statistically different between vitamin C-treated and untreated groups. At D1 after MI and before vitamin C administration, a non-significant difference between patients to be treated by placebo and those to be treated by vitamin C was observed, the latter subset presenting a 11% lower mean value of circulating MP captured onto annexin V (vitamin C 12.45 ± 1.9 vs. placebo 13.9 ± 1.9 nmol L⁻¹ PhtdSer Eq, *P* = 0.57). This slight difference probably results from prospective randomization. As the treatments applied were distributed equally between both subsets, they could hardly account for this observation.

Table 3 Cell origin of circulating MP at day 1 after MI in diabetes mellitus (DM)(n=23), dyslipidemic (DL)(n=36) and high-risk (HR) patients (n=38). HV corresponds to healthy volunteers (n=50). No significant difference in the amount of circulating MP appeared at randomization between vitamin C and placebo group. Circulating MP were captured on antibodies to platelet (GPIb) or endothelial (CD31) membrane antigens. Procoagulant phospholipid content was measured by functional prothrombinase assay, and expressed as nanomolar phosphatidylserine equivalents (nmol L⁻¹ PhtdSer Eq)

	Circulating microparticles during myocardial infarction (day 1) (nmol PhtdSer Eq)				
	Whole population	Diabetes mellitus	Dyslipidemia	High risk factors	Healthy volunteers
Microparticles captured onto:					
Annexin V	13.2 ± 1.85	15.7 ± 3.66	13.4 ± 2.15	13.4 ± 1.82	2.3 ± 0.2
GPIb antibody	3.2 ± 0.54	3.05 ± 0.73	2.98 ± 0.62	2.33 ± 0.52	0.58 ± 0.10
CD31 antibody	0.48 ± 0.20	0.55 ± 0.30	0.40 ± 0.18	0.42 ± 0.14	0.02 ± 0.006

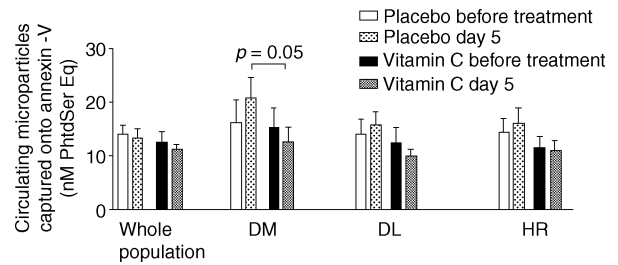


Fig. 1. Circulating procoagulant microparticles levels at day 5 following Myocardial Infarction in vitamin C and placebo groups. Whole population (vitamin C: n = 29; placebo: n = 32), diabetes mellitus (DM + vitamin C: n = 12; DM + placebo n = 11), dyslipidemic (DL + vitamin C: n = 18; DL + placebo: n = 18), high-risk patients (HR + vitamin C: n = 20; HR + placebo: n = 18). Microparticles were captured onto annexin V. MP procoagulant phospholipid content was measured as nanomolar phosphatidylserine equivalents (nmol L⁻¹ PhtdSer Eq) by functional prothrombinase assay.

Circulating procoagulant MP during myocardial infarction

With respect to values measured in HV, patients with MI presented high levels of procoagulant MP by capture onto annexin V (MI 13.2 ± 1.85 vs. HV 2.3 ± 0.2 nmol L⁻¹ PhtdSer Eq). Circulating MP were mainly of platelet (MI 3.2 ± 0.54 vs. HV 0.58 ± 0.10 nmol L⁻¹ PhtdSer Eq) and endothelial (MI 0.48 ± 0.20 vs. HV 0.02 ± 0.006 nmol L⁻¹ PhtdSer Eq) origin. Values of circulating MP levels according to various risk factors are shown in Table 3.

Effect of additional vitamin C treatment on procoagulant MP during MI

In the whole subset of patients with MI, the additional treatment by vitamin C resulted in a slight (~14%) decrease in procoagulant MP captured onto annexin V, while an ~4% reduction was evidenced in the placebo group (Fig. 1). MP measurements in treated or untreated patients, did not reach statistical difference at D5 (vitamin C 10.8 ± 1.3 vs. placebo 13.3 ± 1.7 nmol L⁻¹ PhtdSer Eq). Interestingly, a significant difference was evidenced between untreated and vitamin C-treated DM patients who presented lower MP levels at D5 (DM + vitamin C 12.7 ± 2.6 vs. DM + placebo 20.8 ± 3.8 nmol L⁻¹ PhtdSer Eq; *P* = 0.05). Dyslipidemic and patients at high risk (HR) followed a similar pattern although to a lesser extent (DL + vitamin C 9.9 ± 1.3 vs. DL + placebo 15.7 ± 2.5 nmol L⁻¹ PhtdSer Eq;

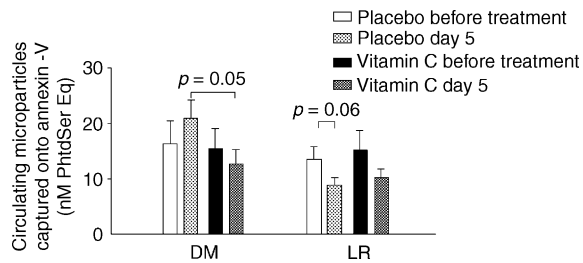


Fig. 2. Circulating procoagulant microparticles levels at day 5 following Myocardial Infarction in diabetes mellitus and low-risk patients (LR). Diabetes mellitus (DM + vitamin C: $n = 12$; DM + placebo: $n = 11$), low-risk patients (LR + vitamin C: $n = 13$; LR + placebo: $n = 10$). Microparticles were captured onto annexin V. MP procoagulant phospholipid content was measured as nanomolar phosphatidylserine equivalents (nM PhtdSer Eq) by functional prothrombinase assay.

$P = \text{ns}$ (HR + vitamin C 11.0 ± 1.8 vs. HR + placebo 16.2 ± 2.5 nmol L^{-1} PhtdSer Eq; $P = \text{ns}$)(Fig. 1). In these two subsets of patients, placebo administration was associated with an $\sim 12\%$ elevation in MP levels captured onto annexin V, to be compared with the 28% elevation measured in DM patients. Interestingly, in patients at lower risk (LR), circulating procoagulant MP at D5 were reduced to the same extent in the presence or absence of additional vitamin C treatment, suggesting its inefficiency on MP release (placebo -34% and vitamin C -31.9%)(Fig. 2).

Effect of additional vitamin C treatment on platelet-derived MP during MI

Platelet-derived MP levels measured after capture onto GPIIb antibody appeared decreased in vitamin C-treated patients regardless of the risk factor (vitamin C 2.2 ± 0.4 vs. placebo 3.6 ± 0.5 nmol L^{-1} PhtdSer Eq, $P = 0.01$). However, the extent of the reduction varied with the clinical background. In untreated DM and HR patients, platelet-derived MP levels showed an $\sim 43\%$ drastic increase after 5 days of placebo administration reflecting an ongoing process of platelet stimulation (HR + vitamin C 1.7 ± 0.3 vs. HR + placebo 3.9 ± 0.7 nmol L^{-1} PhtdSer Eq; DM + vitamin C 3.0 ± 0.6 vs. DM + placebo 4.3 ± 1.1 nmol L^{-1} PhtdSer Eq) (Fig. 3). In patients at lower risk (LR), neither placebo nor vitamin C administration led to elevated platelet-derived MP; on the contrary, a reduction was observed in both cases, the difference between both subsets being non-significant (LR + vitamin C 3.37 ± 0.73 vs. LR + placebo 3.22 ± 0.38 nmol L^{-1} PhtdSer Eq, $P = \text{ns}$).

Effect of additional vitamin C treatment on endothelial-derived MP during MI

Within the whole population of MI patients, a reduction of $\sim 65\%$ in circulating endothelial-derived MP, captured onto anti-CD31 antibody, was evidenced after vitamin C treatment whereas only an $\sim 29\%$ decrease could be evidenced after 5 days of placebo administration. Nevertheless, endothelial-derived MP levels measured in treated and untreated patients

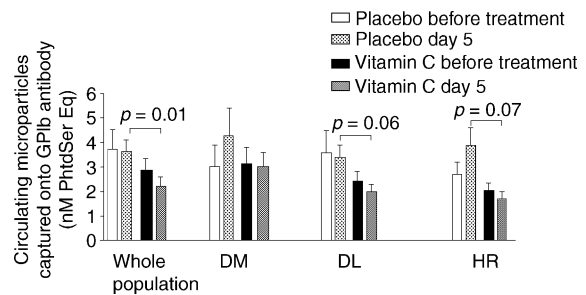


Fig. 3. Platelet-derived microparticles levels at day 5 following Myocardial Infarction in vitamin C and placebo groups. Whole population (vitamin C: $n = 29$; placebo: $n = 32$), diabetes mellitus (DM + vitamin C: $n = 12$; DM + placebo: $n = 11$), dyslipidemic (DL + vitamin C: $n = 18$; DL + placebo: $n = 18$), high-risk patients (HR + vitamin C: $n = 20$; HR + placebo: $n = 18$). Microparticles were captured onto anti-GPIIb antibody. MP procoagulant phospholipid content was measured as nanomolar phosphatidylserine equivalents (nM PhtdSer Eq) by functional prothrombinase assay.

did not reach statistical significance at D5 (vitamin C 0.14 ± 0.02 ; placebo 0.39 ± 0.18 nmol L^{-1} PhtdSer Eq). In HR or DL patients significantly lower levels were, however, detected upon vitamin C treatment (DL + vitamin C 0.12 ± 0.03 vs. DL + placebo 0.59 ± 0.3 nmol L^{-1} PhtdSer Eq; $P = 0.05$; HR + vitamin C 0.11 ± 0.03 vs. HR + placebo 0.56 ± 0.28 nmol L^{-1} PhtdSer Eq; $P = 0.04$). In addition, a drastic elevation ($\sim 60\%$) in circulating endothelial-derived MP was measured in DM patients at D5 after placebo administration, whereas an $\sim 70\%$ reduction was observed after vitamin C treatment (DM + vitamin C 0.12 ± 0.03 vs. DM + placebo 0.97 ± 0.7 nmol L^{-1} PhtdSer Eq; $P = 0.06$)(Fig. 4). As observed for platelet-derived MP, a reduction in endothelial-derived MP was conversely found in LR patients, regardless of the treatment, 67% and 50% , respectively, for vitamin C or placebo, the difference between the two subsets being non-significant (LR + vitamin C 0.15 ± 0.02 vs. LR + placebo 0.35 ± 0.19 nmol L^{-1} PhtdSer Eq, $P = \text{ns}$).

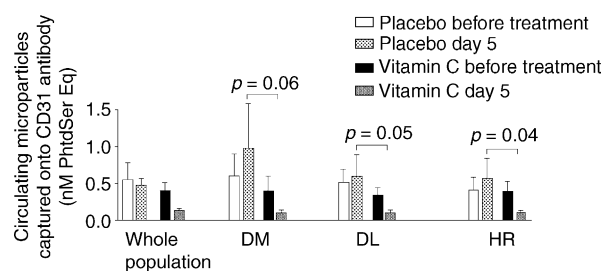


Fig. 4. Endothelial-derived microparticles levels at day 5 following Myocardial Infarction in vitamin C and placebo group. Whole population (vitamin C: $n = 29$; placebo: $n = 32$), diabetes mellitus (DM + vitamin C: $n = 12$; DM + placebo: $n = 11$), dyslipidemic (DL + vitamin C: $n = 18$; DL + placebo: $n = 18$), high-risk patients (HR + vitamin C: $n = 20$; HR + placebo: $n = 18$). Microparticles were captured onto anti-CD31 antibody and their procoagulant phospholipid content measured by functional prothrombinase assay as nanomolar phosphatidylserine equivalents (nM PhtdSer Eq).

Discussion

In patients with MI taken as a whole population, the benefit of additional oral vitamin C treatment could appear modest with a 14% decrease in circulating procoagulant MP levels compared to the 4% reduction in patients with conventional treatment. These measurements, performed after capture onto immobilized annexin V, might underestimate MP populations presenting a proportion of oxidized phospholipids restricting binding to annexin V [24]. However, this limitation appears negligible in view of MP levels measured in clinical subsets in which high oxidative stress may account for enhanced cellular activation. A significant elevation in circulating MP captured onto annexin V was detected in DM as well as the clear decrease upon vitamin C treatment. Furthermore, the inhibitory effect of vitamin C on procoagulant MP release was not observed in patients at lower risk (Fig. 2). In LR patients, amounts of circulating procoagulant MP were decreased to the same extent 5 days after vitamin C or placebo administration. These opposite responses to vitamin C treatment observed in DM and LR patients could reflect a specific contribution of oxidative stress to vascular cell stimulation and MP release in HR patients. In LR patients, oxidative stress might be overwhelmed by other stimuli such as cytokines, shear stress, thrombin and tissue factor occurring, generated or expressed during MI [25]. These observations confirm procoagulant MP as a relevant parameter to follow an ongoing process of vascular damage during MI in patients at high thrombotic risk.

In accordance with previous reports from our group and others, platelet- and endothelial-derived MP appear to be the two main sources of procoagulant MP released during MI [6,7]. Unexpectedly, additional vitamin C treatment resulted in a 14% decrease in platelet-derived MP levels in spite of anti-platelet and antithrombin therapy. In DM and HR patients, although the reduction after 5 days of vitamin C administration could appear modest *per se* (3% and 10%, respectively), it has to be compared to the dramatic elevation in MP levels measured after placebo administration (up to 44%). Thus, vitamin C prevents an ongoing process of platelet activation and membrane shedding in patients at HR, especially in DM.

Diminished platelet aggregation and adhesion by vitamin C has been evoked in previous studies [10,26,27]. In patients with chronic heart failure, vitamin C enhanced platelet responsiveness to the anti-aggregatory effects of NO donors, reduced plasma lipid-derived free radicals and improved endothelial function [28]. Various mechanisms may contribute to these observations: (i) the formation of guanylate cyclase activation and cGMP formation, a potent platelet inhibitor [29]; and (ii) inhibition of inflammatory platelet activating factor mimetics preventing the formation of platelet-leukocytes aggregates and platelet activation [30,31].

Although representing a smaller proportion, endothelial-derived MP appeared highly susceptible to vitamin C treatment. We previously showed that circulating endothelial-derived MP testify to endothelial activation and/or apoptosis [7,32]. Indeed, low levels of circulating endothelial cells were report-

ed in acute coronary syndrome [33], pointing to underlying endothelial apoptosis. In our study, endothelial-derived MP were decreased by vitamin C treatment in DM, HR and DL patients (70% reduction in DM), suggesting a major reduction in endothelial damage (Fig. 4). In DM, results are in accordance with the beneficial effects of vitamin C previously demonstrated on endothelial function [18,34]. Increased apoptosis and high oxidative stress are two features of DM [35]. Various serum factors, namely oxLDL, reactive oxygen species (ROS), angiotensin II, hyperglycemia-mediated superoxide induced endothelial cell apoptosis through enhanced intracellular oxidative stress could be responsive to the treatment [29]. In patients with congestive heart failure, vitamin C and carvedilol were both found to reduce endothelial cell apoptosis, circulating levels of MP, and markers of oxidative stress [23,32].

The drastic effect of additional vitamin C treatment on endothelial-derived MP measured in DM patients emphasizes the specific role of endothelial apoptosis induced by ROS in such a pathology. As an observation added in proof, no modification in leukocyte-derived MP could be evidenced with respect to values measured in HV (data not shown). In DM, ROS could contribute to endothelium dysfunction by reducing bioavailability of NO [36,37], a potent mitochondrial membrane stabilizer [38,39], crucial for endothelial survival. ROS could also promote the release of mitochondrial cytochrome c and caspase activation [40].

Shed MP originating from apoptotic endothelial cells or activated platelets are not only considered markers of vascular damage but also behave as cellular effectors disseminating proinflammatory, pro-adhesive, pro-apoptotic and prothrombotic potentials in the vasculature [41–45]. Recently, various phospholipids borne by MP shed from apoptotic endothelial cells were found susceptible to oxidation and able to elicit specific responses by vascular cells [24,46]. Furthermore, MP isolated from patients with MI selectively impair the endothelial NO transduction pathway [47]. Each MP population released into the blood flow may have a specific contribution to the process of MI, which remains to be characterized. Likewise, the susceptibility of membrane phospholipids and cells to oxidative stress probably differs with the lineage, explaining the variety of the vascular responses [48]. These observations lead to consider procoagulant circulating MP as an eventual target for a pharmacological control in patients at high thrombotic risk.

Our data suggest that an early additional antioxidant treatment may improve endothelial function particularly in subsets of patients in which high oxidative stress was previously demonstrated, such as DL and DM groups. According to the current understanding, vitamin C could promote an early improvement of the cellular redox imbalance and prevent NO inactivation in the vascular compartment. The associated reduction in platelet activation, as evidenced by MP measurements, although less sensitive to antioxidant treatment, could also prove beneficial in patients at higher thrombotic risk.

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