Clinical Research

Acute Administration of Vitamin C Prolongs the Duration of Forearm Reactive Hyperemia in Patients with Hypercholesterolemia

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Introduction: Vitamin C improves endothelial function in patients with hypercholesterolemia. However the effect of vitamin C on reactive hyperemia (RH), which is believed to be at least partly endothelial-dependent, is not known. Aim of this study was to investigate the effect of vitamin C on forearm RH of patients with hypercholesterolemia.

Methods: Forty-six patients (pts.) treated for hypercholesterolemia (diet and/or statins) participated in the study. Strain gauge venous occlusion plethysmography was used to measure forearm blood flow (FBF). FBF was measured in all subjects at rest and during RH. In order to induce RH a cuff inflated at suprasystolic pressure was used to occlude blood circulation at wrist level. FBF during RH was measured every 15 sec for 3 min after release of the cuff. Arterial blood pressure was measured at rest and every min during RH. Subsequently pts. received either 2 gr of vitamin C orally (N=25, 21 males) or placebo (N=21, 19 males) and FBF measurements at rest and during RH were repeated 2 hours later. The degree of RH, as the percent increase of FBF during RH, duration of RH, as well as forearm vascular resistance (FVR) at rest and at RH were calculated.

Results: Administration of placebo did not change significantly FBF at rest (4,9±0,4 vs 4,8+/-0,4 mL· min^{-1}· 100 mL^{-1}, ns) and during RH, the degree (72,7+/-44% vs 93,3±7 %, ns) as well as, the duration of RH (50,7±39 vs 67,8+/-54 sec, ns) and finally the FVR at rest and during RH (27,3±9 vs 28,4±12 and 18,3±9 vs 16,0±9 U, respectively, ns). Vitamin C did not change significantly FBF at rest (5,2±0,4 vs 4,7±0,3 mL· min^{-1}· 100 mL^{-1}, ns) and during RH, the degree of RH (85,0±35% vs 87,4±69 %, ns) and FVR at rest and during RH (29,5±3 vs 29,3±3 U and 14,9±6 vs 16,8±8 U, respectively, ns). Vitamin C did prolong the duration of RH (73,8±57 vs 106,8±55 sec, p=0,05).

Conclusion: Admistration of vitamin C did not prolong the degree of RH, which is considered to reflect structural lesions at microcirculation. It did improve the duration of RH. This supports the opinion that RH is at least partly endothelial-dependent. It is believed that the duration of RH depends partly on the vasodilation induced by the increased blood flow. It is known that increased shear stress due to increased blood flow induces nitric oxide (NO) production by endothelial cells. NO is considered to be the major vasodilator factor produced by endothelial cells. Vitamin C may act as an antioxidant at the level of vascular endothelium by increasing NO bioavailability, which is decreased in pts. with hypercholesterolemia.

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Reactive hyperemia (RH) is a protective mechanism that has been developed in mammals to ensure rapid restoration of the blood flow and the metabolism, whenever the blood flow is abruptly interrupted and ischemia is caused. Myogenic, neurogenic, local metabolic factors as well as the vascular

Table 1. Group A: Subjects who received vitamin C. Group B: Subjects who received placebo. No significant differences were observed between the two groups concerning age and lipid profil.

	Group A	Group B	
N	25	21	
Age (years)	58.7±8	55.8±12	ns
Total cholesterol (mg/dl)	232.9 ± 46	237.0 ± 52	ns
LDL – Cholesterol (mg/dl)	156.5 ± 45	168.5 ± 56	ns
HDL – Cholesterol (mg/dl)	48.7 ± 15	49.5 ± 15	ns
Triglycerides (mg/dl)	151 ± 64	121,1±35	ns
Smokers	3	6	ns
Diabetes Mellitus	6	2	ns
Arterial Hypertension	14	8	ns
Coronary Artery Disease	13	12	ns
Statins	13	13	ns

endothelium are believed to interact, contributing to the manifestation of RH[1-10]. The quantitative contribution of each individual factor depends on the part of the vascular network that is examined, the mammal that is studied and the way with which ischemia is caused. We know that the endothelium plays a part in vascular tone modulation through vasodilating and vasoconstricting factors[11]. The role of the endothelium in the creation of RH has been studied in animals[5-8], while in man's forearm it is not yet completely known[9,10]. It is believed that the endothelium participates in the onset of RH through the production of prostaglandins[3,4,7,8] adenosine[4] and mainly through NO[9,10,12], which is considered to be the main vasodilating factor produced by the endothelium. Studies have shown that there is an interaction between NO and the local metabolites, which promotes the manifestation of RH[6,13,14].

We already know from studies with pharmacological endothelium stimulation that endothelial function in the micro-circulation of the forearm of patients with hypercholesterolemia is disordered[15-19]. However, the effect of hypercholesterolemia on the mechanical stimulation of the endothelium of the forearm arterioles has not been sufficiently studied and there are conflicting indications as far as its influence is concerned on the maximum hyperemic flow[10,15,20-23], while no data are reported with regard to its effect during reactive hyperemic flow. The administration of vitamin C, that is the most potent, water-soluble anti-oxidant in human plasma, has been shown to improve endothelial dysfunction in hypercholesterolemic patients who were not on statins[24]. Up to date there are no data on the effects of vitamin C in RH.

The aim of this study was to investigate the effects of per os administration of vitamin C on the reactive hyperemic flow of the forearm in patients with hypercholesterolemia.

Material

Forty-six patients with hypercholesterolemia (ages 26 to 78 years old, mean age 51.3±8) participated in the study (Table 1). The research protocol was approved by the hospital's ethics committee, it was explained in detail and all patients gave their consent. We must note that although all patients were on a hypolipidemic diet, 26 out of 46 were on statins monotherapy and were not successfully treated. We randomized the patients in to two groups. Group A consisted of 25 patients (21 men), mean age 58.7±8 years, who received vitamin C. Their lipidemic profile was (x±SD): triglycerides 151.0±64 mg/dl, total cholesterol 232.9±46 mg/dl, LDL-cholesterol 156.5±45 mg/dl and HDL-cholesterol 48.7±15 mg/dl. Six patients suffered from diabetes mellitus, 13 from coronary artery disease, 14 from idiopathic arterial hypertension and 3 were smokers. Group B consisted of 21 patients (19 men), mean age 55.8±12 years, who received placebo. Their lipidemic profile was (x±SD): triglycerides 121.1±35 mg/dl, total cholesterol 237.0±52mg/dl, LDL-cholesterol 168.5±56 mg/dl and HDL-cholesterol 49.5±15 mg/dl. Two patients suffered from diabetes mellitus, 12 from coronary artery disease, 8 from idiopathic arterial hypertension and 6 were smokers. Thirteen patients from each group were on statins. Both groups were comparable as far as the number of smokers, diabetic patients, hypertensive patients and patients suffering from coronary artery disease. None of the patients who participated in the protocol suffered from malabsorption syndrome, nor did they take any vitamin supplements or any other anti-oxidants.

Method

All measurements were performed in the morning, before the administration of drugs and at least 8 hours after food intake, with the patients in supine position, completely at rest and after they had been familiarized with the area and the laboratory temperature (25˚C to 26˚C). Their right hand was placed at the supine position on two supporting rubber pillows at the height

of the elbow and the wrist. The forearm blood flow (FBF) was measured first at rest and then during RH. Group A patients then received 2 gr. of vitamin C per os in effervescent tablets and group B patients received placebo in the same form. FBF measurements were repeated two hours later, at rest and during RH. Arterial pressure was measured on the left hand at rest and during hyperemia, every one minute (0, 1, 2 minutes). All patients were instructed not to consume food, beverages, drugs or smoke in between the two hours.

Measurements were taken with the strain gauge venous occlusion plethysmography, using mercury leads (model EC5R, Hokanson DE)[63]. The forearm flow plethysmography with mercury leads is a non invasive, simple and accurate method for the calculation of the forearm FBF. The blood flow was calculated in mL· min^{-1} per 100 mL of forearm or as a percentage of relative change, in order to be able to compare the results of individuals of different size. RH was induced with a 4 minute application of a cuff inflated at suprasystolic pressure to occlude blood circulation at the wrist. FBF measurements during RH were performed 5 seconds after the removal of the cuff and every 15 seconds until the completion of 3 minutes.

Based on FBF at rest and the maximum FBF that corresponds to the first recording in the RH phase, we calculated the RH degree, according to the formula: [(Maximum FBF - Basic FBF) / Basic FBF)] · 100. The RH duration for each group was set as the first recording of FBF during RH that did not have any significant statistical difference from the basic FBF. The vascular resistance of the forearm (FVR) was calculated for every time point as the quotient of FBF to the respective mean arterial pressure that was calculated based on the known formula (Mean arterial pressure = Diastolic arterial pressure + (Systolic arterial pressure – Diastolic arterial pressure) / 3)).

Vitamin C dosage was selected taking in account that the per os administration of 2 gr. of vitamin C causes 2.5 times increase of its concentration in plasma, 2 hours after its administration. This concentration is within the normal values range (30 to 150 μmol/L), and is maintained up to 5 hours after administration[5,62]. This dosage was proven sufficient to improve the endothelial malfunction in patients with coronary artery disease[25].

Statistical analysis

In order to compare the two groups as far as demographics, lipidemic profile, hemodynamic parameters before and after the intervention, we used the paired samples and the independent samples T-test. In order to compare consecutive flow measurements and calculated parameters, we used the one-way ANOVA (between groups analysis) method and the general linear model for repeated measurements (within groups analysis). We used LSD post hoc test for meta-analysis.

The statistical significance of the change during RH for each group was calculated as follows: The mean difference between the basic FBF and the FBF at the time when RH returned to baseline was calculated for every group. We then calculated the duration of RH for every patient independently. We considered that RH stopped at the time when the FBF of the RH was less than the sum of baseline FBF plus mean difference between baseline FBF and FBF at the time when the RH returned to baseline for the whole group. Paired t-test within groups was used to compare the duration of RH before and after the intervention.

Results

The two groups (vitamin C and placebo) had comparable values before the intervention regarding FBF at rest (5.2±0.4 vs. 4.9±0.4 mL· min^{-1}· 100 mL^{-1}, ns), the degree of RH (85.0±35% vs. 72.7±44%, ns), the duration of RH (73.8±57 vs. 50.7±39 sec, ns) and the baseline FVR at rest and during RH (29.5±3 vs. 27.3±2 and 14.9±6 vs. 18.3±9 U, respectively, ns), (Table 2). The administration of placebo did not significantly change FBF at rest (4.9±0.4 vs. 4.8.0.4 mL· min^{-1}· 100 mL^{-1}, ns), the degree of RH (72.7±44% vs. 93.3±7 %, ns), the duration of RH (50.7±39 vs. 67.8±54 sec, ns) and the FVR at rest and during RH (27.3±9 vs. 28.4±12 and 18.3±9 vs. 16.0±9 U, respectively, ns), (Figure 1). The administration of vitamin C did not change FBF at rest significantly (5.2±0.4 vs. 4.7±0.3 mL· min^{-1}· 100 mL^{-1}, ns), the degree of RH (85.0±35% vs. 87.4±69 %, ns) and the FVR at rest and during RH (29.5±3 vs. 29.3±3 U and 14.9±6 vs. 16.8±8 U, respectively, ns), (Figure 2). However, it significantly prolonged the RH duration (73.8±57 vs. 106.8±55 sec, p=0.05), (Figure 3).

Discussion

Reactive hyperemia and endothelium

RH is believed to be divided in two phases, on the basis of the physiological mechanisms that parti-

Table 2. Results: Administration of placebo did not change significantly FBF rest, the degree of RH, the duration of RH, and FVR at rest and at RH. Administrarion of vitamin C also did not change significantly FBF rest, the degree of RH, and FVR at rest and at RH. But it did change the duration of RH(*).

	Group A before vit. C	Group A after vit. C	Group B before placebo	Group B after placebo	
N	25	25	21	21	
FBF at rest ($mL \cdot min^{-1} \cdot 100\ mL^{-1}$)	5.2±0,4	4.7±0,3	4.9±0,4	4.8±0,4	ns
FVR at rest (U)	29.5±3	29.3 ± 3	27.3 ± 9	28.4 ± 12	ns
FVR in hyperemia (U)	14.9 ± 6	16.8 ± 8	18.3 ± 9	16.0 ± 9	ns
Degree of RH (%)	85.0 ± 35	87.4 ± 69	72.7 ± 44	93.3 ± 74	ns
RH Duration (sec)	73.8 ± 57*	106.8 ± 55*	50.7 ± 39	67.8 ± 54	ns

cipate in its manifestation: the early phase and the late phase. The early phase of RH corresponds to the first plethysmographic recording of FBF, 5 seconds after the removal of the cuff. Authors have suggested that myogenic mechanisms[1,2] and local metabolites[3,4,7] that are produced during ischemia cause the vasodilation in the early phase of RH. The role of NO is still under investigation; however, recent studies claim that NO participates slightly in this procedure[9,10]. In this phase, the highest possible FBF is observed as well as the lowest possible FVR, that is the maximum vasodilating capacity of arterioles, that also constitutes an indicator of the structural changes in micro-circulation[21,26]. In the present study, the early phase is expressed through the RH degree. We deemed that the expression of the RH degree, as a percent of change compared to FBF at rest is more reliable as an indicator than the absolute value of maximum FBF-that has been used in the past[20-22]. The percent age of change allows us to compare patients groups regardless of the baseline FBF, that may be different. In this specific study, baseline FBFs of both groups were different, however their difference did not reach levels of statistical significance. The role of the endothelium, probably through NO production, is believed more important in the late phase of RH[9-10]. The forearm blood flow increase leads to mechanical stimulation of the endothelium and to NO production, due to increase of the shear strength. In the present study, the late phase of RH is expressed through its duration. In the studies up to date, greater emphasis has been given to early phase RH recording[20-22]. Studies in hypercholesterolemic populations have shown conflicting results regarding the disorder of the maximum vasodilating capacity of the forearm's vessels[20-22], possibly due to the presence of different

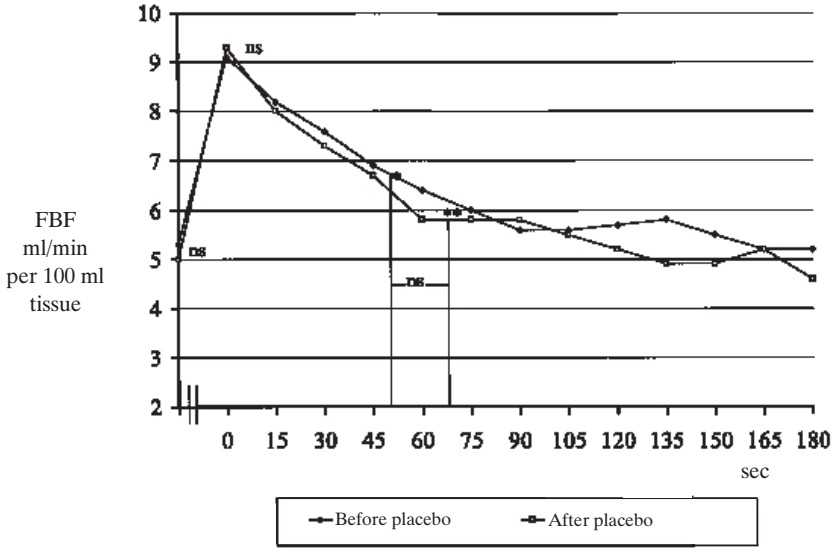

Figure 1. FBF in group B before and after placebo. FBF at rest, FBF during RH as well as duration of RH did not differ significantly before and after placebo administration.

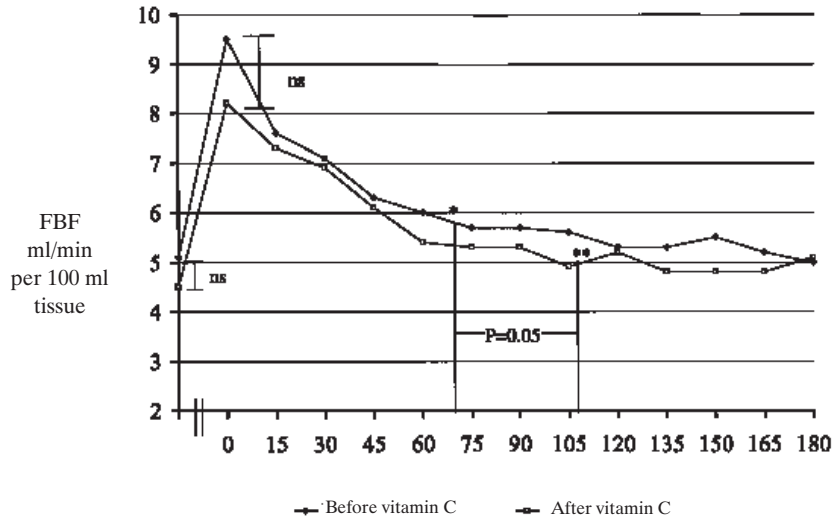

Figure 2. FBF in group A before and after vitamin C. FBF at rest and FBF during RH did not differ significantly before and after vitamin C administration. Duration of RH was significantly increased after vitamin C administration (**) compared to duration of RH before ascorbic acid administration (*).

degree subclinical atherosclerotic lesions in the peripheral vessels of the populations used in each study. No studies have been conducted until now regarding RH duration in patients with coronary artery disease or with coronary artery disease risk factors.

Conclusions

Vitamin C administration did not influence the RH degree-early phase, that is related with the maximum vasodilation of the peripheral vessels and constitutes an indicator of atherosclerotic lesions. On the contrary, it significantly prolonged RH duration-late phase. The percentage of change of FBF was comparable in both groups (vitamin C group and placebo group), thus, the mechanical stimulation of the endothelium through application of shear strength was comparable too. It seems that vitamin C improved the phase of RH that is believed to be endothelium-dependent, maybe by increasing NO bioavailability. This finding is compatible with the aspect that in the early phase of RH, myogenic and local metabolites have the principal role and **not** the endothelium.

Hyperlipidemia and endothelium

In hypercholesterolemic patients, we have found that there is a malfunction of the vascular endothelium both in coronary circulation[36-38], as well as in the forearm microcirculation[15-19]. The increase of the oxidative stress seems to be a common and basic mechanism through which hypercholesterolemia and other risk factors (arterial hypertension, diabetes mellitus, smoking) lead to endothelial dysfunction[30]. It has been argued that the increased production of

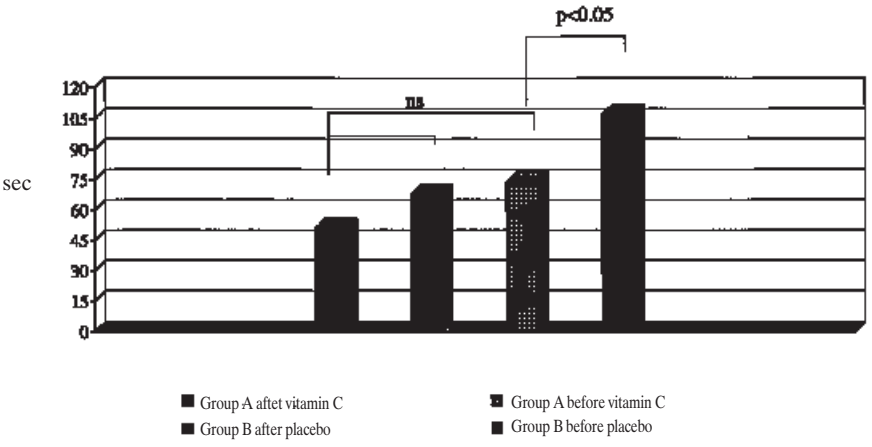

Figure 3. Duration of RH in group A and B before and after vitamin C and placebo administration respectively.

peroxide anions[31], as well as the imbalance of the intracellular anti-oxidative system of glutathione[32], that are observed in the vascular tissue of hypercholesterolemic patients, lead to reduced NO bioavailability. Increased NO destruction by the peroxide free radicals further leads to increased production of peroxynitrite ions[33], an endothelium toxic radical.

The role of vitamin C in the vascular endothelium

Vitamin C constitutes the most potent, water-soluble, anti-oxidant in human plasma[34-37]. Several studies have indicated that acute administration of vitamin C improves the endothelial function in healthy smokers[38], as well as in hypertensive patients[41,42], patients suffering from diabetes mellitus[43], hypercholesterolemia[33], coronary artery disease[25,39,44], and heart failure[45]. Many different mechanisms have been considered regarding the way with which vitamin C improves endothelial function[46]. Two are the mechanisms that have been considered most probable in the above mentioned studies: a) neutralization of oxygen free radicals, such as peroxide anion[47-51] and b) the interaction with glutathione intracellularly, regulating in this way the intracellular oxidation-reduction balance[51-53].

In the present study, the acute administration of vitamin C was per os, in contrast with most of the previous studies, where the administration was intra-arterial or intravenous[24,38-45], with just one exception[25]. The concentration of vitamin C in the plasma of individuals who do not take vitamin dietary supplements ranges from 30 to 60 μmol/L[54]. The expected concentration of vitamin C following administration of 2 gr per os is approximately double than the initial one[25]. According to a recent study, vitamin C concentration of 100 μmol/L in the plasma is not considered sufficient to give a reaction with the peroxide anions due to the reaction constant (3.3×10^5 $\mu mol \cdot L^{-1} \cdot s^{-1}$) that is approximately 10^5 times smaller than the constant of the reaction of the peroxide anions with NO[56]. The concentration of vitamin C in plasma required to neutralize the superoxide anions would be at the level of 10 mmol/L , which could only be achieved parenterally. On the other hand, the active transfer of vitamin C in the cells[57] leads to intracellular concentration reaching 6-8 mmol/L[58-60]. This concentration would be enough to neutralize superoxide anions intracellularly[56]. Despite the fact that vitamin C has been considered as a first line of defense against free radicals in plasma[61], its beneficiary action in this specific case seems to be related with the change of the intracellular oxidation-reduction potential[35,62].

Consequently, the acute administration of vitamin C cannot improve the already existing anatomical-structural disorders in the forearm microcirculation, as one would expect. The improvement of atherosclerotic lesions has been proven feasible in the forearm microcirculation with chronic treatment with statins[21]. It may be that chronic co-administration of anti-oxidative substances can lead to further improvement. However, acute administration of vitamin C improved the endothelium-dependent phase of RH in the forearm of hypercholesterolemic patients. It is important that vitamin C action was achieved with per os administration, i.e. with normal plasma levels of vitamin C. The RH duration, as described in the present study, may constitute a new method for the study of the endothelium, the first one that will study the integrity of endothelial function in micro-circulation through mechanical stimulation.

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