

Antioxidant Vitamin Levels Do Not Exhibit Negative Correlation with the Extent of Acute Myocardial Infarction

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Summary

Serum levels of vitamin E (VE), β -carotene (BC) and vitamin C (VC) were determined in 50 patients with the first acute myocardial infarction (AMI) before starting thrombolytical treatment. VE and BC were determined by HPLC, VC spectrophotometrically. The reperfused patients were divided according to vitamin concentrations into four groups. The lowest quartile was compared with the rest of the studied population (VE: group with high (H) $> 15.6 \mu\text{M}$ $>$ group with low (L), BC: H $> 0.07 \mu\text{M}$ $>$ L, VC: H $> 25 \mu\text{M}$ $>$ L) in the following parameters: extent of myocardial damage (area under the curves of troponin I, CK-MB during 48 h), arrhythmia and congestive heart failure occurrence, size of ejection fraction, positivity of ventricular late potentials. No significant differences between groups H and L for either VE, BC or VC were found ($P > 0.05$). As no correlation between serum concentrations of vitamins E, C and β -carotene and the extent and clinical course of AMI was found, the actual vitamin concentrations may be important for prevention of ischemic heart a disease, but they do not play a decisive role in the acute phase of myocardial infarction in humans.

Key words

Antioxidant vitamins • α -Tocopherol • β -Carotene • Ascorbic acid • Myocardial infarction.

Introduction

The development of techniques, which restore the flow of oxygenated blood to ischemic myocardial tissue, has led to major advances in the treatment of acute myocardial infarction (AMI). However, it has been recognised that the process of myocardial reperfusion

itself may lead to a number of adverse consequences including reversible contractile dysfunction – myocardial stunning, reperfusion arrhythmia, endothelial dysfunction and even cell death. Several mechanisms may participate in these processes, the production of reactive oxygen species (ROS) in myocardial tissue being of special importance (Young *et al.* 1993).

During thrombolytical therapy of AMI, when reperfusion takes place after initial hypoxia or even anoxia, substantial amounts of ROS are formed, which initiate the cascade of lipoperoxidation. Membrane destruction occurring during reperfusion correlates with the duration of reperfusion, extent of neutrophil accumulation and myocardial contractile dysfunction (Chen *et al.* 1995). The generation of free radicals poses no problem so far as it is in balance with their eradication either by enzyme activity or by natural antioxidants. As far as the non-enzymatic defense system is concerned, the antioxidant activity was found to be exhibited especially by vitamin E, C, β -carotene, glutathione, uric acid, albumin, cysteine, ceruloplasmin and bilirubin.

In our previous study (Mužáková *et al.* 2001) an increase of the serum malondialdehyde, stable product of lipoperoxidation, and a decrease of the serum level of vitamin E and β -carotene in patients with AMI was found shortly after thrombolytic treatment. The enzymatic part of the defence system, namely glutathione peroxidase in whole blood, was found to be stimulated during reperfusion (unlike the erythrocyte superoxide dismutase) (Mužáková *et al.* 2000). As we found that the pool of antioxidant vitamins is gradually depleted during elimination of reactive oxygen species during reperfusion treatment of AMI, we decided to verify the hypothesis that vitamin shortage can contribute to clinical complications of AMI.

Table 1. Group characteristics

No. of patients	50
Age	61.1 \pm 10.6
Male/female	36/14
Body mass index (kg/m ²)	27.7 \pm 4.0
Positive family history (%)	24.0
Total cholesterol (mmol/l)	6.1 \pm 1.0
HDL (mmol/l)	1.3 \pm 0.4
Triacylglycerols (mmol/l)	2.7 \pm 1.7
Index athero (Klimov)	4.3 \pm 1.6
Fibrinogen (g/l)	2.8 \pm 0.9
Diabetes mellitus (%)	16.7
Hypertension (%)	56.2
Cigarette smoking (%)	50

Patients and Methods

Subjects

Characteristics of the studied group of patients are given in Table 1. Fifty patients (36 men, 14 women) in an acute phase of myocardial infarction treated by thrombolytical therapy (streptokinase: Streptase, Behringwerke Co., Germany, 1.5 MIU/1 h) fulfilled the following criteria: the first myocardial infarction (chest pain of more than 20 min duration, electrocardiographic changes consistent with AMI: pathological Q or at least 2 mm of ST segment elevation in two precordial or two inferior leads), admission within 6 h after the onset of AMI, successful thrombolysis (maximum of CK-MB within 12 h after the administration of thrombolytic therapy).

Other treatment was given as required. Patients who had other serious health complications were excluded. A written informed consent was obtained from all the participants before starting the protocol and the study was approved by the Hospital Ethical Committee on Human Research. All patients underwent twelve-lead electrocardiography and transthoracic echocardiography. Echocardiography was performed on the third day after AMI. Left ventricular ejection fraction (EF) was used as a parameter in the assessment of myocardial function. Incidence of arrhythmias within 48 h after AMI and positivity of ventricular late potentials on electrocardiographic curve were also evaluated. The congestive heart failure (Killip-Kimball) occurrence was followed during the first 48 h after AMI. The extent of myocardial damage was determined as the area under the curves of CK-MB and troponin I during 48 h (Mair *et al.* 1995).

Methods

Peripheral venous blood samples were obtained from each patient just before the beginning of the therapy. Serum samples were collected into tubes containing 1,4-dithioerythritol (antioxidant), immediately frozen and stored at -70 °C. Other blood samples for creatine phosphokinase MB and troponin I determinations were collected immediately after admission and after 1.5, 3, 6, 12, 24 and 48 h after onset of therapy. The determination was performed with commercial Creatine kinase MB isoenzyme kit (Dade Co., USA) using analyser Dimension RxL, Dade Behring (USA) and with kit Immulite Turbo Troponin I using analyzer DPC Immulite (USA).

Vitamin E and β -carotene were analyzed with

HPLC (Ecom, CR) as described by Mužáková *et al.* (2001). Serum vitamin C concentrations were measured by the photometric method based on the oxidation of ascorbic acid to dehydroascorbic acid, which forms a colored product with 2,4-dinitrophenylhydrazine in the

presence of thiourea (absorbance at 560 nm) with spectrophotometer Agilent 8453 (Agilent Technologies, Germany).

Statistical methods used were t-test, nonparametric test of two samples, NCSS2000.

Table 2. Correlation matrix of antioxidant levels (vitamin E, VitE/chol, VitE/chol+TAG, β -carotene, Vitamin C) and size of myocardial damage (CK-MB Area).

	Vitamin E	VitE/chol	VitE/chol+TAG	β -carotene	Vitamin C
<i>VitE/chol</i>	0.856				
	0.000				
<i>VitE/chol+TAG</i>	0.724	0.924			
	0.000	0.000			
<i>β-carotene</i>	0.127	0.184	0.213		
	0.400	0.221	0.155		
<i>Vitamin C</i>	0.056	0.111	0.100	0.238	
	0.709	0.461	0.509	0.112	
<i>CK-MB area</i>	-0.024	-0.064	-0.003	-0.084	0.163
	0.873	0.672	0.983	0.579	0.280

Data represent significance levels (p values).

Table 3. Comparison of mean values and standard deviations in patient groups with high levels of α -tocopherol (AT), β -carotene (BC) and vitamin C (VC) with the lowest quartile.

	AT < 15.6 $\mu\text{mol/l}$	AT > 15.6 $\mu\text{mol/l}$	BC < 0.07 $\mu\text{mol/l}$	BC > 0.07 $\mu\text{mol/l}$	VC < 25 $\mu\text{mol/l}$	VC > 25 $\mu\text{mol/l}$
<i>Myocardial damage</i>	94.5 \pm 64.5	89.3 \pm 63.2	105.1 \pm 58.9	85.5 \pm 64.4	66.4 \pm 44.5	99.1 \pm 67.1
<i>Arrhythmia occurrence</i>	38 %	32 %	39 %	32 %	33 %	34 %
<i>Congestive heart failure II/III</i>	31 % / 8 %	32 % / 3 %	39 % / 8 %	30 % / 3 %	25 % / 0	38 % / 5 %
<i>Ejection fraction</i>	47.7 \pm 11.1	52.0 \pm 11.9	50.2 \pm 11.4	51.1 \pm 11.9	50.3 \pm 14.1	51.1 \pm 10.8
<i>Positivity of ventricular late potentials</i>	33 %	24 %	30 %	25 %	15 %	26 %

No significant differences ($p < 0.05$) were found.

Results

In order to evaluate the potential correlation of the serum levels of different antioxidants with the extent of myocardial damage we constructed a correlation matrix of individual parameters (Table 2). Correlation analysis did not reveal any significant relationship between the main determined parameters. The only significant correlation was found between the levels of vitamin E and vitamin E expressed per cholesterol amount and/or per the sum of cholesterol and triacylglycerols. As no correlation was found between the antioxidant levels and the size of myocardial damage, we hypothesized that only the lack of vitamins below the threshold limit may exhibit deleterious effects. To verify this hypothesis the patients were divided into four quartiles according to the vitamin levels. The group corresponding to the lowest quartile was compared in the followed parameters with patients above the threshold (the cutting point for the lowest quartile of the studied population).

The α -tocopherol level in the studied population varied in the range 7.9-44.5 $\mu\text{mol/l}$, the mean concentration being 19.7 ± 7.2 $\mu\text{mol/l}$ and the threshold value 15.6 $\mu\text{mol/l}$. The comparison of the AMI clinical course markers in the group with lower (L group) and higher levels (H group) of α -tocopherol is shown in Table 3. None of the determined parameters showed a significant difference between the groups, either when the level of α -tocopherol was expressed in relation to cholesterol or to the sum of lipids.

Comparison of the groups in risk factors of ischemic heart disease did not reveal significant differences in age, occurrence of diabetes mellitus and/or hypertension, body mass index, HDL or triacylglycerol concentration. Low α -tocopherol levels correlated with the lower concentration of cholesterol (L: 5.5 mmol/l vs. H: 6.3 mmol/l, $p < 0.05$). In the group with lower concentration of α -tocopherol stronger cigarette smokers were found (L: 13.7 cigarettes/day vs. H: 5.5 cigarettes/day, $p < 0.05$).

The β -carotene level in the studied population varied in the range 0.03-0.56 $\mu\text{mol/l}$, the mean concentration was 0.09 ± 0.07 $\mu\text{mol/l}$ and the threshold value 0.07 $\mu\text{mol/l}$. The comparison of the AMI clinical course markers is shown in Table 3. As in the case of α -tocopherol, no statistically significant difference between the L group and H group was detected.

The group comparison did not reveal significant

differences in monitored risk factors of ischemic heart disease (IHD) with the exception of cigarette smoking. Again cigarette smokers had a significantly lower concentration of the monitored vitamin (L: 13.9 cigarettes/day vs H: 4.8 cigarettes/day, $p < 0.05$). This finding corresponds to the higher oxidative stress and thus higher antioxidant consumption in case of cigarette smoking.

Wide range of vitamin C levels was detected in the studied population (11-92 $\mu\text{mol/l}$), the mean value being 44.4 ± 21.4 $\mu\text{mol/l}$ and the threshold value 25 $\mu\text{mol/l}$. Neither in this case was correlation found between clinical markers and vitamin level (Table 3). The group comparison did not reveal significant differences in any of the monitored risk factors of IHD.

Discussion

In animal models ROS were detected directly in myocardial tissue and the extent of free radical production was shown to correlate with the incidence of reperfusion arrhythmia and myocardial stunning (Bernier *et al.* 1986). Enhanced ROS production was proved both directly using electron paramagnetic resonance spectroscopy in patients with AMI (Grech *et al.* 1996) and indirectly by assaying the myocardial content of malondialdehyde after AMI (Young *et al.* 1993).

A variety of pharmacological compounds have been investigated for their ability to limit the extent of reperfusion injury, in particular oxygen free radical scavengers, antioxidants, but also calcium channel blockers, inhibitors of neutrophils, nitric oxide etc. Antioxidant studies have given rather contradictory results probably due to the variation in degree of collateral blood flow, duration of ischemia, drug delivery method or timing of drug administration. Drugs given intravenously during ischemia may not reach the ischemic area in the absence of collaterals. For this reason it is important to focus our attention to the endogenous substances which participate in diminishing of the negative impact of ischemia-reperfusion injury and are present in the organism in sufficient amounts before the onset of ischemia. The present study has been focused on antioxidant vitamins. Cardioprotective effect of antioxidants, e.g. vitamin E, has been repeatedly demonstrated. For verification of the hypothesis that antioxidant vitamin shortage contributes to ischemia-reperfusion injury we tested the relationship between the extent of myocardial damage and the level of vitamin E,

C and β -carotene. We did not find any statistically significant correlation. These results are contradictory to several studies, especially those using animal model systems. Tripathi and Hegde (1997) reported that pre-treatment with α -tocopherol decreased the size of myocardial infarction and the relative extent of myocardial necrosis in dogs. Vitamin E supplementation led to the decrease of ROS production in rabbit mitochondria (Scholz *et al.* 1997) as well as to an improvement of hemodynamic function in rabbits with AMI (Palace *et al.* 1999). Dietary supplementation of vitamin E contributed to the improvement of mammalian heart antioxidant capacity. Carrasquedo *et al.* (1999) found negative correlation in humans between the extent of myocardial infarction and the level of vitamin E but not of β -carotene. They did not find any correlation between the vitamin levels and congestive heart failure. Riemersma *et al.* (2000) found a correlation between low levels of vitamin C and ventricular tachycardia and fibrillation occurrence, but not with the extent of myocardial infarction. Vallance *et al.* (1978) found negative correlation between the serum levels of vitamin C and the size of myocardial infarction. Polidori *et al.* (2002) reported that lower plasma levels of α -tocopherol, β -carotene and other antioxidant micronutrients are inversely correlated with the severity of heart failure and the ejection fraction in patients with congestive heart failure. Negative correlation was found between the level of malondialdehyde and the ejection fraction in patients with congestive heart failure (Polidori *et al.* 2002). In an extensive overview of 39 studies focused on the effect of antioxidants, 19 of them reported a positive effect on myocardial function, while in 20 of them no improvement was found (Bolli *et al.* 1991).

Taking into account discrepancy of the results of individual studies, Spencer *et al.* (1999) formulated a hypothesis that only the lack of vitamin E, i.e. its concentration below the threshold, represents a risk factor of ischemic heart disease. Supplementation above this limit does not provide any additional protective effect. To verify this hypothesis, we divided the patients according to serum levels of individual vitamins into four groups and compared the lowest quartile with the rest of the studied population. However, the comparison of myocardial damage markers (area under the curves of CK-MB and troponin I) as well as of clinical markers (arrhythmia and congestive heart failure occurrence, size of ejection fraction and positivity of ventricular late potentials) did not show any significant differences

between groups with higher and lower concentrations either of α -tocopherol, β -carotene or of vitamin C.

In our study we detected a broad range of arrhythmias within 48 h after initiation of thrombolytic therapy. Besides usually determined ventricular arrhythmias, ranging from premature beats, ventricular tachycardia and accelerated idioventricular rhythm to ventricular fibrillation, we also observed AV block and bradycardia. The association between ROS and arrhythmogenesis was demonstrated in a number of electrophysiological studies with free radical generating systems. ROS were found to induce changes in membrane action potential characteristics. Oxidative stress may modify the activity of a number of ion-transporting proteins in the sarcoplasmic reticulum and sarcolemma causing intracellular calcium overload, oscillatory release of Ca^{2+} from the sarcoplasmic reticulum, and potentially arrhythmogenic oscillations in the membrane potential (Ošťádal and Kolář 1999). In animal models the administration of agents with an antioxidant effect, as e.g. superoxide dismutase, catalase, ascorbate or glutathione, protected myocardium against reperfusion-induced arrhythmias, while the situation in humans seems to be much more complicated.

Besides clinical markers we also followed classical risk factors of ischemic heart disease. In accordance with the results of Opltová and Hlúbik (1996) we found positive correlation between the level of α -tocopherol and concentration of total cholesterol. This lipophilic vitamin is bound to lipoproteins in the plasma. In accordance with Škrha *et al.* (2003) we expressed its concentration in relation to the sum of lipids (concentration of total cholesterol and triacylglycerols). However, neither in the case of standardized α -tocopherol a significant correlation was found in main parameters. In the group with lower α -tocopherol and lower β -carotene we found significantly more smokers. This finding is in accordance with the results of Stegmayr *et al.* (1993) and Gokkusu *et al.* (2001), as well as those of Street *et al.* (1994), who found positive correlation between the low level of β -carotene and increased risk of AMI in cigarette smokers. Cigarette smoke contains many oxidants, which cause damage of the epithelium of respiratory passageways and are partially absorbed into the blood circulation. This caused fast oxidative breakdown of α -tocopherol and β -carotene (Handelman *et al.* 1996). Thus cigarette smoke increases oxidative stress as well as stimulates catabolism of antioxidants.

In conclusions, no correlation was found among

serum concentrations of vitamin E, β -carotene and vitamin C and the extent of myocardial infarction and clinical course of AMI. Discrepancy between the results of studies on animal models or *in vitro* cultures and clinical trials may be due to a different status of myocardium at the beginning of the studies. Infarction manifestation in humans is usually connected with advanced atherosclerotic changes of coronary arteries, which had been developing for a prolonged period. A wide range of pathological processes contributes to the AMI manifestation. Antioxidant vitamins affect oxidative stress, which represents just one of many simultaneous

processes involved in the progression of the disease. Antioxidant vitamins, according to our results, do not seem to play a decisive role in the acute phase of myocardial infarction. However, their permanent intake is necessary during the whole lifespan to minimize the development of atherosclerotic changes, which are substantially potentiated by ROS.

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