Indian J Physiol Pharmacol 2007; 51 (2) : 165–169

CHANGES IN VITAMIN C AND VITAMIN E DURING OXIDATIVE STRESS IN MYOCARDIAL REPERFUSION

REEMA SOOD*, A. P. S. NARANG*, REBECCA ABRAHAM**, UMA ARORA**, RAJNEESH CALTON*** AND NITIN SOOD****

*Department of *Biochemistry,*
Dayanand Medical College & Hospital, Ludhiana – 141 001
*Departments of **Biochemistry and ***Cardiology,*
Christian Medical College & Hospital,
Ludhiana – 141 008
and
*Department of ****Medicine,*
B.J.S. Dental College, Hospital and
Research Institute, Ludhiana

(Received on February 28, 2006)

Abstract : Injury to the myocardial tissue due to ischemia and reperfusion occurs because of imbalance between the formation of oxidants and available antioxidants in the heart. Levels of vitamin C (ascorbic acid) and vitamin E (∝ - tocopherol) were evaluated in 52 patients of acute myocardial infarction (AMI) treated by streptokinase. They were further divided into reperfused group (39 patients) and non-reperfused group (13 patients). Twenty normal healthy subjects served as controls. Vitamin C and vitamin E were estimated in study group before and after thrombolytic therapy and in controls. Vitamin C levels were low in AMI cases as compared to controls (8.74 ± 1.87 and 10.63 ± 3.26 mg/L, respectively, P<0.001). Trend of fall in vitamin C levels in the two study groups was not statistically significant. Vitamin E levels declined from 12.19 ± 6.71 to 9.96 ± 6.50 mg/L by 4 hours which was significant (P<0.01) in the reperfused group, but the change in non-reperfused group (9.28 ± 6.37 to 9.35 ± 6.07 mg/dL by 4 hours) was non-significant. This is because of increased consumption of this antioxidant in suppressing the oxidative stress which occurs with reperfusion. Vitamin E can be proposed as a valid marker for reperfusion.

Key words : ∝ - tocopherol, ascorbic acid, acute myocardial infarction, reperfusion

INTRODUCTION

Early treatment by thrombolytic therapy is essential for the survival of the ischaemic myocardium to improve venticular function, reduce myocardial necrosis and mortality. Paradoxically, restoration of blood flow may by itself increase the severity of tissue injury termed as reperfusion injury (1, 2). Several studies have shown that reactive oxygen

*Corresponding Author : Dr. A.P.S. Narang, Professor of Biochemistry, Dayanand Medical College & Hospital, Ludhiana – 141 001

species (ROS) play a crucial role in its pathogenesis (3, 4, 5) leading to deleterious physiological consequences of venticular arrythmias, depressed contractile function and lethal myocyte injury (6).

The damage caused by ROS is generally contained by a synergistic, multilevel defence antioxidant system (7) - the enzymatic part and non-enzymatic part. The latter includes a large number of natural or synthetic antioxidant compounds (eg. Vitamin E and vitamin C) which have the ability to inhibit the oxidative damage by scavenging the highly destructive free radical species (8). They may be overwhelmed under pathological conditions. In recent times, attention has been given to the concept of reducing myocardial injury at the time of reperfusion by pretreatment with free radical scavenger vitamins (9, 10). In evaluating such treatment, there is a need to monitor the actual levels of vitamins to assess the status of these available antioxidants.

Vitamin E is the major lipid soluble antioxidant present in blood, mainly in VLDL and LDL fractions, where it prevents free radicals to oxidize these lipoproteins. When incorporated into membranes, it protects myocardial phospholipids (11). In addition to this, Vitamin C acts as the first line of defence against oxidative stress during ischemia reperfusion cycle (12). It is the only antioxidant in plasma capable of completely inhibiting oxidative modification of LDL by aqueous peroxyl radicals (13). Vitamin C administration exerts a protective role against peroxidative damage of lipids (14).

Direct detection of ROS is complicated by their highly reactive and transient nature. Indirect evidence for the presence of free radicals has been demonstrated by the estimation of markers of oxidative stress like antioxidant vitamins –E and C levels during ischemia and after reperfusion.

Our aim was to assess the changes in plasma concentrations of vitamin E and C levels after AMI and thrombolytic therapy and to evaluate the diagnostic utility of these parameters as early non-invasive markers of oxidative stress.

METHODS

Fifty-two patients clinically diagnosed of myocardial infarction presenting within six hours of onset of symptoms constituted the study group. All of them received 1.5 million units of streptokinase intravenously over 60 minutes as per established protocols. A detailed clinical history was sought from each patient including consumption of drugs and exogenous vitamins. Patients who had a history of prior vitamin intake were excluded from the study. They were further divided into 2 groups – reperfused (39 cases) and non-reperfused (13 cases) depending upon the clinical criteria of reperfusion which include : (i) Relief of chest pain (ii) Reversion of ECG changes and reperfusion arrythmias (if any) (iii) CPK rise (iv) Infarct related artery patency (wherever delayed angiogram was done). Twenty healthy adults with matched age and sex having no history or clinical evidence of acute or chronic stress, who were non-smokers, non alcoholics and not on any medications including vitamins constituted the control group.

Venous blood was drawn from controls and patients at three different time intervals

0 hour – baseline i.e. before thrombolysis, 2 hours and 4 hours after thrombolytic therapy. Vitamin E and C were estimated in patients and controls. Vitamin E was estimated by a spectrophotometric method (15). Vitamin C was estimated in plasma by titration method using 2,6-dichlorophenolindophenol (16).

Statistical Analysis was performed using two sample t test or Mann Whitney U-statistics. The patient subgroups were compared by employing Repeated Measured Analysis of Variance to test the time hypothesis/trend. All analysis was performed using PC 90 (Dixon Brown, 1990) BMDP and SAS (SAS Inc, Gary, North Carolina) U.S.A. Software Libraries.

RESULTS

Levels of Vitamin C and vitamin E are represented in Table I. Levels of Vitamin C were significantly lower in AMI patients as compared to controls ($P<0.05$) while those of Vitamin E were non-significant ($P>0.05$). Comparing the two study groups, vitamin C levels at all time intervals of the study were lower in the reperfused group ($P<0.05$) but were not different from the non-reperfused group (Table II). The decrease in vitamin E levels in the reperfused group was statistically significant as compared to the decrease in non-reperfused group which is non-significant (Table III).

TABLE I: Mean±S.D. levels of study parameters of acute myocardial infarction patients before thrombolytic therapy compared to healthy controls.

Parameter	*AMI (n=52)*	*Controls (n=20)*	*P value*
Vitamin C (mg/L)	8.74±1.87	10.63±3.26	0.05*
Vitamin E (mg/L)	11.45±6.69	10.25±4.06	0.52

TABLE II: Vitamin C levels (mg/L) at different time intervals before and after thrombolytic therapy in acute myocardial infarction.

Group	*0 hours*	*2 hours*	*4 hours*
Reperfused (n=39)	8.6±1.95	8.23±1.76	7.93±1.91
Non-reperfused (n=13)	9.13±1.62	8.80±1.61	8.26±1.44

TABLE III: Vitamin E levels (mg/L) at different time intervals before and after thrombolytic therapy in acute myocardial infarction.

Group	*0 hours*	*2 hours*	*4 hours*
Reperfused (n=39)	12.19±6.71	11.46±6.71	9.96±6.50
Non-reperfused (n=13)	9.23±6.37	9.76±6.49	9.35±6.07

DISCUSSION

This study documented significantly lower levels of vitamin C in AMI cases as compared to the control groups which is comparable to other studies (17, 18, 19). It has been recently demonstrated that during reperfusion of the ischemic heart, there is increased generation of oxygen free radicals (4, 20) and depletion of endogenous antioxidants (19). Significantly lowered levels of vitamin C compared to healthy individuals can be due to its enhanced consumption due to increased oxidant stress caused by free radicals. Evidence of protective effects of vitamin C in reperfusion injury has been provided by many investigators (21, 22). These provide indirect evidence of reduced

Vitamin C levels after myocardial reperfusion.

No change in vitamin C levels till 24 hours after reperfusion was reported by Young et al (4). They suggested that Vitamin C release accompanies catecholamine release from adrenal medulla (where both are stored). This counteracts the fall in Vitamin C owing to oxidative stress, thus preserving plasma Vitamin C levels. Dusinovic 1998 (23) noted decrease at different time intervals after thrombolytic therapy which was not significant statistically.

Although the levels of vitamin E were higher in patients of AMI as compared to controls, the difference was not statistically significant. The present study has highlighted a significant fall in vitamin E levels in both the reperfused and the non-reperfused groups at 0, 2 and 4 hours post thrombolytic therapy. These observations are in agreement with earlier studies (4, 23, 24). The decreased levels have been explained on the basis of consumption of the membrane free radical scavenger in presence of excess oxygen free radicals formed during reperfusion.

Other investigators have provided indirect evidence of reduced vitamin E levels after reperfusion by highlighting the beneficial effects of supplementation of this membrane stabilizer in both animal models and human studies (25, 26, 27). There is however no previous reports on elevated levels of Vitamin E before and after reperfusion. An interesting observation in the current study is the significant difference between the reperfused and the non-reperfused groups with regard to the decline of vitamin E levels. This confirmed the fact that increased free radical generation which follows resumed blood flow results in increased consumption of vitamin E and hence lower levels in reperfused patients as compared to those non-reperfused. Thus it can be considered as a suitable, valid early non-invasive marker for reperfusion.

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