The State of Lipid Peroxidation and Antioxidants Following Thrombolytic Therapy with rt-PA and Streptokinase in Acute Myocardial Infarction

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SUMMARY

The role of reactive oxygen products in myocardial damage caused by ischemia-reperfusion has been established in a number of studies performed in animals models. However, studies showing the development of increased free radicals following effective myocardial reperfusion in humans are scarce. In the present study, both the increase of lipid peroxidation (LPO) following early stage thrombolytic therapy which is the current treatment issue performed after acute myocardial infarct (AMI) and the plasma levels of vitamin E and C (chain braker antioxidants) were investigated parallel to time.

Forty patients with AMI who were admitted to hospital within six hours from the beginning of symptoms were included in the study and divided into two groups; group 1 (recombinant tissue-Plasminogen Activator, rt-PA group) and group 2 (streptokinase group). Serial serum specimens were drawn before and 30, 90 minutes and 24 hours after thrombolytic therapy for the investigation of LPO, vitamin E and C levels. Echocardiographic examination was performed on the tenth day to evaluate the functions of the left ventricle. Plasma levels of lipid peroxides (LPO) were found to increase 90 minutes after thrombolytic therapy in each group, while the levels of vitamins E and C showed significant decreases. The difference between the two groups was not significant. Similar to this finding, no significant difference in the ejection fraction values was observed between the groups. Further, no correlation was observed between the ejection fraction and LPO values at the 90th minute which is considered to be the time of successful thrombolysis.

In conclusion, the occurrence of a series of biochemical changes confirming an increase in free radical development of peripheral blood was observed. Although the decrease in vitamin E and C levels suggests the need for supplementation of these vitamins along with the thrombolytic therapy, the fact that at least a week is needed for an increase of tissue levels of vitamin E confirms

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the opinion that the daily prophylactic doses of these vitamins is suitable for the decrease of AMI risk. (Jpn Heart J 1999; 40: 267–273)

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THE use of thrombolytic therapy in the treatment of acute myocardial infarction (AMI) has produced several benefits such as a significant increase of the recovery of left ventricular function.^{1–3)} There is a direct correlation between the elimination of coronary artery obstruction following thrombolysis and the enhancement of left ventricular functions.⁴⁾ However, some studies have demonstrated that, partial dysfunction in the left ventricle may persist in the months following thrombolytic therapy.⁵⁾ This condition has been attributed to the reperfusion injury due to thrombolysis. Particular attention has been given to the production of large amounts of oxygen radicals from the onset of reperfusion, which may be responsible, at least in part, for irreversible peroxidative damages to myocardial tissue.²⁾ Several biochemical mechanisms may contribute to an explosive increase in free radical production in the reperfused myocardium which can overwhelm cellular and extracellular free radical scavenging systems, leading to myocardial and endothelial dysfunction.¹⁾ Experimental studies on animals have proved the role of free radicals in ischemia-reperfusion injury. The evidence supporting the presence of increased free radical production following successful myocardial reperfusion in man is more limited.¹⁾ Albeit studies demonstrating that the increase in lipid peroxidation markers following coronary artery balloon angioplasty for the therapy of stabilized angina and the production of free radicals as a consequence of reperfusion are isochronized^{6,7)} the number of studies analysing the increased lipid peroxidation following thrombolytic therapy, and the state of the antioxidants during this period, with relation to time, are few.¹⁾ Some workers have found a drop in plasma antioxidant vitamins in patients with AMI.⁸⁾ If occlusion / reperfusion causes significant consumption of antioxidants after myocardial infarction, then a greater fall might be expected in patients with thrombolytic therapy.⁹⁾ Therefore, measurements of antioxidants over a longer time period might give more direct evidence of the severity of the oxidative stress during ischemia and reperfusion.

The aim of this study was to investigate the modification in the levels of vitamins E and C, which are antioxidants, parallel to the possible increase in lipid peroxidation markers, following thrombolytic therapy with two different thrombolytic agents rt-PA and streptokinase in patients with AMI, in relation to time.

PATIENTS AND METHODS

Forty patients with AMI who entered to the Ege University Hospital, Emergency Cardiology Unit 6 hours following the onset of symptoms were included in the study. Two groups were formed:

Group 1 (rt-PA group): 100 mg of Recombinant Tissue Plasminogen Activator (rt-PA, Actilyse) was administered to 20 patients with AMI, in the form of bolus and infusion in 90 minutes.

Group 2 (streptokinase group): 1,500,000 units of streptokinase were administered to 20 patients with AMI, intravenously over 60 minutes.

In addition to the thrombolytic therapy, support therapy with aspirin (200 mg) and heparin (5000 units bolus + 1000 units/hr infusion) was given to both groups. Serum levels of LPO markers (MDA), vitamin E and vitamin C were detected immediately after (0 minutes) and in 30 minutes, 90 minutes and 24 hours following thrombolytic therapy. Serum samples for LPO detection, which were supplemented with glutathione and EDTA, were stored at -20°C , together with other serum specimens, until they were processed.

LPO was quantified as p-MDA (plasma malondyaldehyde) which is the main TBARS (thiobarbituric acid-reactive substance) in plasma by a method modified from Yagi.¹⁰⁾ The colorimetric determination of vitamin E as total tocopherols in serum was employed according to Martinek.¹¹⁾ In this oxidimetric color reaction, ferrous iron is produced by the reduction of ferric iron by vitamin E in serum.

Vitamin C levels were determined photometrically with 2, 4 dinitrophenylhydrazine to form the red bis-hydrazone, which is reduced to a colorless form.¹³⁾

The Mann-Whitney U-test or unpaired *t*-test was used to assess the significance of differences between results on different times, accepting *p* values of 0.05 as significant. Correlation was determined according to Pearson correlation analysis.

RESULTS

Changes in levels of plasma malondialdehyde and antioxidant vitamins (vitamins E and C) as a function of time from the beginning of thrombolysis with rt-PA and streptokinase for acute myocardial infarction are shown in Tables I and II.

There was no significant difference between the two groups in relation to p-MDA, vitamin E and vitamin C levels at 0 (initial), 30, 90 minutes or 24 hours ($p > 0.05$).

Table I. Results in Patients Treated with rt-PA for Thrombolysis Following AMI

	0 min	30 min	90 min	24 hours	10 th day
TBARS (nmol/ml)	3.973 ± 0.144	4.029 ± 0.177	4.716 ± 0.235*	4.251 ± 0.218	
Vit. E (mg/dl)	1.015 ± 0.101	0.893 ± 0.089*	0.707 ± 0.082*	0.641 ± 0.061*	
Vit. C (mg/dl)	1.186 ± 0.107	0.899 ± 0.099*	0.850 ± 0.059*	0.830 ± 0.110*	
EF (%)					45.95 ± 1.95

* $p < 0.05$ when compared to 0 minutes**Table II.** Results in Patients Treated with Streptokinase for Thrombolysis Following AMI

	0 min	30 min	90 min	24 hours	10 th day
TBARS (nmol/ml)	4.032 ± 0.186	4.021 ± 0.199	4.687 ± 0.340*	5.111 ± 0.326*	
Vit. E (mg/dl)	1.168 ± 0.120	0.994 ± 0.125*	0.875 ± 0.118*	0.755 ± 0.081	
Vit. C (mg/dl)	1.255 ± 0.122	0.986 ± 0.106*	0.924 ± 0.097*	0.929 ± 0.097*	
EF (%)					43.70 ± 1.95

* $p < 0.05$ when compared to 0 minutes

p-MDA levels showed a significant increase at 90 minutes when thrombolysis was achieved, in both groups ($p < 0.05$). These levels decreased to the basal level at 24 hours in the rt-PA group, while the increase persisted in the streptokinase group.

On the contrary, a significant decrease was observed in vitamin E and C levels at 90 minutes ($p < 0.05$), and the decrease progressed till 24 hours. Ejection fraction (EF) values, which indicate left ventricular function, obtained 10 days after the thrombolytic therapy did not show a significant difference between the two groups ($p > 0.05$). Also, there was no significant correlation between the levels of p-MDA at 90 minutes and the EF values in each group.

DISCUSSION

The aim of reperfusion is to reduce the size of the ischemic area. Although necessary to minimize tissue injury of the postischemic myocardium, reperfusion may be associated with several disadvantages, as has been clearly indicated by numerous experimental studies performed in various animal models.²⁾ This adverse effect can be reduced by treatment with various antioxidants at the time of myocardial reperfusion, which indicates that free-radical formation is an important factor in their pathophysiology.⁶⁾ Therefore, it is important to determine whether increased free radical activity occurs following reperfusion of the heart in humans. Malondialdehyde is one of the small-mass compounds resulting from fragmentation of polyunsaturated fatty acids undergoing reactive oxygen species attack, a fact that qualifies this aldehyde as a marker of lipid peroxidation.⁹⁾ In this study, we have demonstrated transient changes in MDA and antioxidants in

peripheral blood following successful thrombolytic therapy with streptokinase and rt-PA in acute myocardial infarction. A decrease in antioxidant levels and a synchronic elevation production in MDA provides strong support for the presence of increased free radical activity. Raised plasma malondialdehyde concentrations have been reported in patients in whom successful myocardial reperfusion was achieved after thrombolytic therapy for acute myocardial infarction. In non-reperfused subjects, no increment was observed.^{1,2,5)} Davies *et al.* demonstrated a rise in p-MDA in a single sample taken 2 hours after the start of administration of streptokinase for acute myocardial infarction.⁵⁾ Giardina *et al.* observed a progressive increase in plasma MDA after thrombolysis only in reperfused patients, whose values at the third, sixth and 24th hours were also significantly greater than those of nonreperfused patients.²⁾ Young *et al.* have found an early rise in plasma MDA levels with peaking at 90 min. accompanied by a reciprocal decrease in alpha-tocopherol and retinol in reperfused patients. Ascorbate levels did not change significantly until 24 h. Where the coronary artery was occluded no changes in these parameters were found except for a late (24 h) fall in ascorbate.¹⁾ Levy *et al.* have recently found an increase in plasma oxidizability and a drop in plasma antioxidant vitamins in patients with acute myocardial infarction throughout streptokinase therapy.⁷⁾ In another study, Pucheu *et al.* demonstrated a significant increase in plasma MDA levels and no significant alteration in the antiradical compounds (Beta-caroten, vitamin A and, uric acid) in all patients who had been administered intravenous thrombolysis.¹³⁾

In this study, we also found a significant increase in plasma MDA levels at 90 min. when thrombolysis was achieved, in patients given streptokinase and rt-PA for acute myocardial infarction. On the contrary, a significant decrease was observed in vitamin E and C levels in 90 minutes. In our study, at 10 days following admission, the left ventricular ejection fraction was also assessed in patients with AMI. There was no significant correlation between the levels of p-MDA at 90 minutes and the EF values in each group nor a significant difference between the two groups. Young *et al.* have not found any relationship between lipid peroxidation and left ventricular ejection fraction,¹⁾ in contrast to the work of Davies *et al.*⁴⁾ Davies *et al.* have found a weak correlation between the rise in thiobarbituric acid reactive materials and left ventricular ejection fraction in patients with a patent artery.^{4,14)} In a previous study, Mickelson *et al.* reported that streptokinase may preserve myocardial and coronary vascular function by scavenging reactive oxygen species.¹⁵⁾ Stringer *et al.* have recently demonstrated that tPA reduced neutrophil O₂ - generation by a mechanism that is not dependent on tPA proteolytic activity and is not a function of direct scavenging.¹⁶⁾

These findings might be helpful in explaining our results; the reason that we

did not observe any difference in p-MDA, vitamin E and vitamin C levels and left ventricular ejection fraction values between the groups that were administered streptokinase and rt-PA could be that both of these agents exert antioxidant effects, streptokinase by directly scavenging reactive oxygen species and rt-PA by reducing the generation of neutrophil O_2^- .

In conclusion, a series of biochemical changes accompanied by an increase in TBARS levels which tend to promote the production of increased levels of free radicals due to reperfusion injury and an opposing decrease in the chain breaking antioxidant (vitamin E and C) levels were observed in patients with AMI, who have been treated with two different thrombolytic agents such as rt-PA and streptokinase.

No significant correlation between the increase in TBARS levels and the EF values has been established. A point to be emphasized is that, EF values obtained at a late period of thrombolysis (10th day), indicate the size of the infarct region, rather than the myocardial injury due to the increase of free radicals. Therefore, it would be more reasonable to look for a correlation between immediate EF values and TBARS levels.

The decreases in vitamin E and C levels point to the possible use of administering these two vitamins, in addition to the thrombolytic therapy. However, since a few weeks are required for vitamin E, which is lipid-soluble, to reach adequate levels in the tissue, the routine daily use of these vitamins may be of value to prevent atherosclerosis and may also prove to be useful as a supplement to thrombolytic therapy in AMI encountered at some time in the future.

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