Antioxidants decrease reperfusion induced arrhythmias in myocardial infarction with ST-elevation

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1. ABSTRACT

In myocardial infarctions with ST-segment elevation, ischemia followed by reperfusion (IR) leads to arrhythmia, myocardial stunning and endothelial dysfunction injury by reactive oxygen species (ROS). To determine the impact of ROS, we examined the effect of antioxidant vitamins on biochemical changes and arrhythmias induced by reperfusion before and after therapeutic thrombolysis (Actilyse®). As compared with those receiving placebo, in individuals who received antioxidants, there was a significant decrease in premature ventricular beats (100% vs 38%), atrial fibrillation (44% vs 6%), ventricular tachycardia (31% vs 0%), first-degree atrial-ventricular block (44% vs 6%), plasma malondialdehyde at the first hour after initiation of thrombolysis ($1.07 +/- 0.10$ vs $0.53 +/- 0.10$ nmols plasma malondialdehyde/mg protein) and circulating neutrophils after 24 hr after reperfusion. The antioxidant capacity of plasma was increased from $1.89 +/- 0.15$ to $3.00 +/- 0.31$ units/mg protein and paraoxonase-1 rose from $0.77 +/- 0.08$ to $1.27 +/- 0.11$ nmol/min/mg protein. These findings suggest that antioxidants might be useful as adjuvants in controlling reperfusion induced arrhythmias following therapeutic alteplase thrombolysis.

2. INTRODUCTION

Rapid restoration of coronary artery patency and salvage of ischemic myocardium are key aims in the management of myocardial infarction with ST-elevation (STEMI). Treatment with intra-venous thrombolytic agents improves both systolic function and survival (1-2). Concomitant with the reperfusion of the ischemic myocardium, however, reactive oxygen (ROS) and nitrogen (RNS) species are rapidly released by the ischemic tissue, endothelium and leukocytes (3-4). These species induce cell injury, and cause endothelial and microvascular dysfunction (5). Processes as lipoperoxidation, accumulation of arachidonic acid (AA) and formation of hydroxylated AA such as 20-hydroxyeicosatetraenoic acid (20-HETE), exert potent vasoconstrictor effect and alter the level of endogenous biomolecules (6). Recruitment, adhesion and activation of neutrophils is enhanced during reperfusion, and contribute to neutrophil-mediated reperfusion injury, by depletion of endogenous antioxidants (7-8). Such an injury is characterized by temporary impairment of systolic function (myocardial stunning), arrhythmias and presumably necrosis of myocardium (9-10).

Under normal conditions, there is a decrease in oxidant production and depeletion of endogenous sources of ROS in ischemic heart (8, 11, 12). The full clinical impact of reperfusion injury has been difficult to assess and might be feasible only by the use of preventive measures such as by decrease of oxidant production or increase in antioxidant defense such as by vitamins E and C, scavengers of oxygen-derived free radicals, inhibitors of ROS generating enzymes, and inhibitors of nucleoside uptake by human forearm muscle, which increase endogenous adenosine levels (7). One such agent, N-acetylcysteine (NAC), which potentiates the hemodynamic and antiaggregative effects of nitroglycerine has already been in clinical use (13). *In vitro*, NAC has been found to scavenge several oxygen-derived free radicals and oxidants, to ameliorate the extent of reperfusion injury in animal models, and result in reduced myocardial stunning (14-16). This finding suggests that anti-oxidants might be useful in clinical treatment of reperfusion induced injury. To assess such an effect, here, we examined the effects of a mixture of anti-oxidants, vitamins, co-factors and minerals in reperfusion induced arrhythmias in myocardial infarction with ST-elevation (17-24).

3. MATERIALS AND METHODS

3.1. Patients

A controlled clinical, blinded, randomized (by aleatory numbers table) study was carried out in 32 patients (5 women and 27 men) admitted to coronary care unit. Inclusion criteria were age $\leq$ 65 years, with onset ischemic chest pain of $\leq$ 6 h duration, electrocardiogram (EKG) evidence of transmural ischemia ($\geq$ 1 mm ST-segment elevation in $\geq$2 limb leads and/or $\geq$ 2 mm ST-segment elevation in $\geq$2 precordial leads), and enzyme activity of creatine kinase total (CK-total) and MB-fraction (CK-MB), aspartate aminotransferase (AST) and lactic dehydrogenase (LDH) in plasma. Exclusion criteria were no reperfusion myocardial (no resolution ST- segment elevation 90 minutes after thrombolysis and Thrombolysis In Myocardial Infarction (TIMI) $\leq$ 1 flow grade), past Q-wave myocardial infarction; previous severe (New York Heart Association Class IV) cardiac failure; systolic blood pressure < 90 mm Hg on admission; hemodynamically significant valvular heart disease; ingestion of allopurinol, antioxidant supplements, penicillamine or gold salts within the previous 7 days; and contraindications to use of alteplase (surgery or cerebrovascular accident within the previous 6 weeks, warfarin therapy, active peptidic ulcer, bleeding disorders, or uncontrolled hypertension, and bacterial endocarditis or acute pancreatitis). Immediately after the diagnosis was made and the central catheter was introduced into the right atrium via the brachial vein, the patient was monitored. Witnessed oral informed consent was requested, confirmed by written informed consent was obtained from all patients as soon as it was possible. This study was carried out after review and approval of the medical ethics committee of the institution.

3.2. Treatment strategy

After obtaining a complete clinical history to record acute or chronic disease, each participant was randomly assigned to one of two groups. The first group (n = 16) received placebo (0.9 % NaCl solution). The second group (n = 16) received capsules with an antioxidant mixture (17) (MAOx, Diabion®, Merck Mexico: vitamin A (1000 µg), vitamin C (100 mg) (18), vitamin E (100 mg) (19), thiamine (5 mg) (20), vitamin B_{12} (5.0 µg) (19), pyridoxine (5.0 mg) (21), magnesium (87.5 mg) (22), zinc (15 mg) (23), folic acid (400 µg) (19), selenium (70 µg) (24) and chromium (70 µg). The MAOx or placebo capsules were administered orally; two capsules were administered together after establishment of diagnosis and initiation of thrombolysis. Other capsules were individually administered, 1, 4 and 12 hr later. Thrombolysis was achieved by 90 min intravenous infusion of <100 mg delivery of Alteplase (Actilyse®).

3.3. Electrocardiographic ST-segment elevation and analysis of arrhythmias

EKGs were performed on admission and 120 minutes after thrombolysis. The sum of ST segment elevation was measured 20 ms after the end of the QRS complex from leads I, aVL and V_1 to V_6 for anterior and leads II, III, aVF and V_5 to V_6 for inferior myocardial infarction. The second EKGs were classified by comparison of the ST segments with those on the first EKGs. Normalised ST segment was defined as no residual ST-segment elevation of 0.1 mV or more in any of the 12 leads (complete ST-segment elevation resolution); improved ST segment was defined as residual ST-segment elevation $\leq$ 70% of that on the first EKG (partial ST-segment elevation resolution). To monitor the incidence of reperfusion arrhythmias were analyzed from continuous recordings of 6 to 12-lead EKGs at speed of 25 mm/s and defined as any arrhythmia that is presented from the beginning, during and up to 120 minutes after thrombolysis. Reperfusion arrhythmias were classified a follows; single premature ventricular beats (PVBs), ventricular tachycardia (VT) was defined as three or more consecutive premature ventricular beats at a rate of >100 beats per minute (bpm), morphologically similar ventricular extrasystoles, ventricular fibrillation (VF) was defined as irregular undulations of varying shape and amplitude on the electrocardiogram without discrete QRS or T waves, resulting in acute hemodynamic compromise requiring cardioversion, atrial fibrillation (AF) was defined as disorder atrial contraction with a frequency that varies among 400 at 700 bpm, sinus bradycardia (SB) was defined as sinus rhythm with a frequency smaller than 60 bpm, first degree atrial-ventricular block (FDAVB) was defined as dysfunction of the conduction of the electric impulse from the sinus node (SN) through the auricles to the atrial-ventricular node (AV) with interval P-R (> 0.20 seconds) and a frequency among 60 - 100 bpm, second degree atrial-ventricular block (SDAVB); type-I Mobitz was defined as dysfunction of the conduction of the electric impulse from AV node with irregular interval P-R until appears wave P without complex QRS; type-II Mobitz was defined as retard in the electric conduction of the node AV with constant interval P-R ($\geq$ 0.20 seconds), third-degree atrial ventricular block (TDAVB) was defined as the block of all the impulses from the NS to node AV, ventricular frequency < 45 bpm with interval P-P regular, interval R-R

regular, there is not relationship among rhythm atrial and ventricular. EKGs were evaluated by an investigator who unaware of the clinic data.

3.4. Coronary angiography and analysis of left-ventricular function

All subjects underwent coronary angiography to evaluate left ventricular ejection fraction and regional dyssynergic area of the left ventricle related to the infarct-related artery was performed after 48 hours thrombolysis, according to the clinical policy of the unit. Angiography was carried out by way of the right femoral artery by the Judkins technique. TIMI flow grades were evaluated by an investigator, unaware of the clinic data or treatment assignment. TIMI 0 was classified by the absence of flow antegrade of the occlusion. TIMI 1 denotes penetration of the contrast agent without perfusion and failing to opacity the entire coronary bed distal to the occlusion. TIMI 2 was defined as partial perfusion with a slower rate of entry or clearance of the coronary bed than in other comparable areas. TIMI 3 flow was defined by complete perfusion with antegrade flow proximal to the obstruction and rapid clearance from an uninvolved bed in the same or opposite coronary artery. Left ventricular ejection fraction was assessed by ventriculography (mapping) in the right and left anterior oblique view (Bicor-PLUS-TOP-System. SIEMENS, Germany).

3.5. Biochemical analysis

Whole blood (1 ml) obtained from the catheter was mixed in a 1.5 ml Eppendorf tube with 10 μl 2mM butylated hydroxytoluene (BTH) and 10 μl sodium heparin. Plasma obtained by microcentrifugation of this mixture was used to assess the antioxidant capacity of plasma (ACP). The total antioxidant efficiency of plasma comprised of both enzymatic and non-enzymatic mechanisms was measured by evaluating the capacity of an aliquot of plasma *in vitro* to inhibit controlled oxidation and molecular breakage by lipoperoxidation (25). To do this, micelles of linolenic and linoleic acids 90 μM were prepared by agitation in 7.2 mmol/l phosphate buffer, pH 8, for 15 s and maintained at 4°C until used. Ten μl aliquots were mixed with 10 μl of plasma, 4 mmol/l H_2O_2, 2 mmol/l $CuSO_4$ (for hydroxyl radical generation), and 10 mmol/l phosphate buffer, pH 7.4, to a final volume of 0.5 ml. The mixture was incubated for 10 min at 37°C. The reaction was stopped by adding 1 ml 0.375% thiobarbituric acid (TBA) in 0.2 N HCl and heating in a steam bath for 15 min. Finally, 0.5 ml of 0.2 N HCl was added to stop the reaction (26). The tubes were cooled and the absorbance was measured at 532 nm (malondialdehyde, MDA). 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, MO) was used as standard. One unit of antioxidant capacity of plasma (ACP) is defined as the amount of plasma necessary to inhibit 50 % of the MDA formed *in vitro* under the specific conditions described above. Protein was measured as reference parameter (27).

The paraoxonase 1 (PON1) activity was measured by adding 20 μl of plasma to 500 μl Tris/HCl buffer (100 mmol/l, pH 8.0) containing 2 mmol/l $CaCl_2$ and 5.5 mmol/l paraoxon (O,O-diethyl-O-*p*-nitrophenylphosphate; Sigma Chemical Co) (28). The rate

of generation of p-nitrophenol was determined at 405 nm, 25°C, with a continuously recording spectrophotometer (Beckman DU 800).

Aliquots of plasma were simultaneously utilized for clinical chemical determinations including automated white blood cells counts (CELL-DYN 3700 Abbott Laboratories. Illinois-USA) and differential leukocyte counts, and enzyme activity of creatine kinase total (CK-total) and MB-fraction (CK-MB), aspartate aminotransferase (AST) and lactic dehydrogenase (LDH) in plasma using an automated analyzer (ILab 600 Instrumentation Laboratory Company, Lexington MA, USA). The plasma MDA concentration was also measured at different times and considered as a biomarker of plasma lipoperoxidation.

3.6. Statistical analysis

Date are expressed as mean ± standard error mean (SEM) or as percentages. Statistical analysis included Student.'s t test, X^2 test, Fisher`s test, analysis of variance (ANOVA) and Tukey's HSD. A p value of <0.05 was considered as being statistically significant.

4. RESULTS

The clinical characteristics of the study subjects are shown in Table 1. The prevalence of STEMI in this study was higher in males (n=27) than females (n=5), and well-known predisposing factors such as diabetes mellitus, arterial hypertension, hypercholesterolemia and smokers were present in more of the 50 % of the patients studied. No statistical differences in any of the clinical characteristics were found between the two randomly-assigned groups.

4.1. Electrocardiographic ST-segment elevation

There was no statistical differences in 50% resolution of partial ST-segment elevation and 50% complete ST-segment elevation in placebo group as compared to those treated with MAOx (43.8% partial ST-segment elevation resolution and 56.2% complete ST-segment elevation resolution) (Table 2).

4.2. Coronary angiography, left-ventricular function and markers of myocardial injury

Coronary angiography revealed TIMI 2 flow in 7/16 (43.8%), and TIMI 3 flow in 9/16 (56.2%) in placebo group. and TIMI 2 flow was 6/16 (37.5%), TIMI 3 flow was 10/16 (62.5%) in subjects treated with MAOx (p=not significant). The left ventricular ejection fraction was respectively 56% and 59% group in placebo and MAOx treated subjects (Table 2).

There was respectively 4.7, 3.4, and 2.4 fold increase in the amount of CK, AST and LDH 12 hr after admission without significant differences between placebo and MAOx treated subjects Table 2).

4.3. Arrhythmias

there was a significant decrease in premature ventricular beats (100% vs 38%), atrial fibrillation (44% vs

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Table 1. Clinical characteristics of the study participants

Characteristics	Control Group (Placebo)	Treated Group (MAOx)	p Value
Age (years)	[1] 61.4±11.7	[1] 58.0±8.7	[2] p = 0.34
Males / Females	13 / 3	14 / 2	[3] p = 1.0
Height (m)	[1] 1.64±0.090	[1] 1.62±0.096	[2] p = 0.67
Weight (Kg)	[1] 76.35±9.7	[1] 68.88±12.5	[2] p = 0.06
Diabetes Mellitus	7 / 16	10 / 16	[4] p = 0.48
Arterial Hypertension	8 / 16	5 / 16	[4] p = 0.47
Smoker	14 / 16	11 / 16	[3] p = 1.0
Body mass index (kg/m^2)	[1] 28.47±0.90	[1] 25.96±0.88	[2] p = 0.06
Hypercholesterolemia	9 / 16	9 / 16	[4] p = 0.72
Previous angina	2 / 16	1 / 16	[3] p = 1.0

Data presented are number patients. 1 = Mean ± Standard error. 2 = Student's t test, 3 = Fisher's test. 4 = X^2 test. n=16 for each independent group. MAOx: Mixture of antioxidant agents.

Table 2. Biochemical and hemodynamic aspects of myocardial infarction

Characteristics	Control Group (Placebo).	Treated Group (MAOx)	p Value
Creatine kinase total UI / L (fraction MB)			
0 hours	[1] 254±52 ([1] 28±5)	[1] 337±49 ([1] 33±7)	[2] NS
1 hours	[1] 529±76 ([1] 69±11)	[1] 586±86 ([1] 70±9)	[2] NS
4 hours	[1] 1050±187 ([1] 157±23)	[1] 1504±157 ([1] 180±20)	[2] NS
12 hours	[1] 1812±267 ([1] 201±43)	[1] 2155±212 ([1] 213±50)	[2] NS
24 hours	[1] 1206±193 ([1] 129±23)	[1] 1371±136 ([1] 148±29)	[2] NS
Lactic dehydrogenase UI / L (LDH)			
0 hours	[1] 204±30	[1] 169±14	[2] NS
1 hours	[1] 279±29	[1] 236±20	[2] NS
4 hours	[1] 376±43	[1] 365±25	[2] NS
12 hours	[1] 609±74	[1] 543±39	[2] NS
24 hours	[1] 504±63	[1] 568±71	[2] NS
Aspartate aminotransferase UI/L (AST)			
0 hours	[1] 63±10	[1] 43±4	[2] NS
1 hours	[1] 118±23	[1] 86±5	[2] NS
4 hours	[1] 187±36	[1] 147±14	[2] NS
12 hours	[1] 276±49	[1] 230±20	[2] NS
24 hours	[1] 217±30	[1] 194±19	[2] NS
Location infarction:			
Inferior	8	12	[3] NS
Anterior	8	4	[3] NS
Time from symptoms onset of AMI Thrombolysis (min)	[1] 230±28.7	[1] 213±23.7	[2] NS
Killip class:			
I	10	11	[3] NS
II	5	4	[3] NS
III	1	1	[3] NS
Left ventricular ejection fraction (%)	[1] 56±15	[1] 59±13	[2] NS
TIMI flow grade			
TIMI 2	7 (43.8%)	6 (37.5%)	[2] NS
TIMI 3	9 (56.2%)	10 (62.5%)	[2] NS
Partial ST-segment elevation resolution	8 (50 %)	9 (43.8%)	[2] NS
Complete ST-segment elevation resolution	8 (50%)	7 (56.2%)	[2] NS
Adjunctive medical therapy within the first 24 h after admission			
Aspirin	16 (100%)	15 (93.7%)	[2] NS
Beta-blockers	5 (31.2%)	4 (25%)	[2] NS
ACE Inhibitors	11 (68.7%)	10 (62.5%)	[2] NS
Low molecular weight heparin	16 (100%)	16 (100%)	[2] NS
Statins	15 (93.7%)	14 (87.5%)	[2] NS

Data are presented as percentages and there corresponding number of patients. 1 = Mean ± Standard error. 2 = Student's t test. 3 = X^2 test. NS: not significant. n=16 for each independent group. MAOx (commercial mixture of antioxidant vitamins, cofactors and minerals)

6%), ventricular tachycardia (31% vs 0%), first-degree atrial-ventricular block (44% vs 6%) in placebo as compared with MAOx treated subjects (Table 3). However, the MAOx group showed a significant increase in sinus bradycardia in 8/16 (50%) of patients compared with those who received placebo. None of the MAOx patients showed ventricular tachycardia.

4.4. Biochemical parameters

Among the biochemical parameters measured, the total leukocyte and neutrophil counts were statistically different before and after thrombolysis (Figure 1). In the placebo group, leukocyte count increased from 7.3 to 9.0 x 10^9/l at 24 hr while during the same duration, these values decreased significantly (10.5 ± 7 to 8.13 ± 4 x 10^9/l) in the MAOx patients (p < 0.01). The neutrophil count in the MAOx patients fell from 8.7 ± 7 to 5. 6 ± 4 x 10^9/l after 24 h reperfusion. This decrease was more in the MAOx group than in the placebo (p < 0.0001).

In the placebo group, there was a higher MDA concentration peak (1.36 ± 0.31 nmols MDA/mg protein)

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Table 3. Arrhythmias in the first 2 hours after thrombolysis

Arrhythmias	Patients Number with arrhythmias (Group Placebo)	Patients percentages with arrhythmias (Group Placebo)	Patients Number with arrhythmias Treated Group (MAOx)	Patients percentages with arrhythmias Treated Group (MAOx)	p Value
Atrial Fibrillation (AF)	7	44	1	6	[1] < 0.05
First-Degree A-V Block (FDAVB)	7	44	1	6	[1] < 0.05
Left Bundle Branch Block (LBBB).	3	44	4	25	NS
Premature Ventricular Beats (PVBs)	16	100	6	38	[2] < 0.001
Right Bundle Branch Block (RBBB).	5	31	2	13	NS
Second-Degree A-V Block (SDAVB)	1	6	2	13	NS
Sinus Bradycardia (SB)	1	6	8	50	[1] < 0.05
Third-Degree A-V Block (TDAVB)	2	13	1	6	NS
Ventricular Fibrillation (VF)	3	19	1	6	NS
Ventricular Tachycardia (VT)	5	31	0	0	[1] < 0.05

Data are presented as percentages and there corresponding number of patients. 1= Fisher's test. 2= X^2 test. n=16 for each independent group. Atrial-ventricular (A-V). NS: not significant, MAOx: Mixture of antioxidant agents. Presence of multiple arrhythmias in a single patient can not be excluded
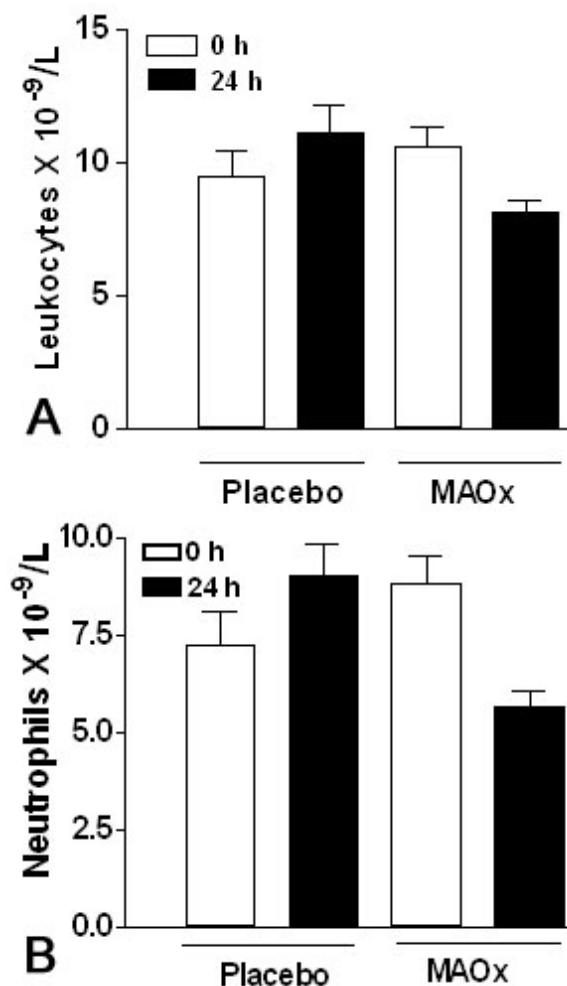

Figure 1. Total leukocyte and neutrophils counts before thrombolysis (white bars) and 24 hours after reperfusion (black bars). The two bars of the left are the placebo group and the treated group is shown on the two bars of the right in 1-a and 1-b. The counts of neutrophils increase significantly after thrombolysis ($p < 0.05$) (Paired t test). The number of both total leukocytes and neutrophils measured decreases significantly in the treated group 24 hours after reperfusion ($p < 0.01$) (Paired t test). This response is different when placebo group was compared to the MAOx group ($p < 0.01$) (Student's t test).
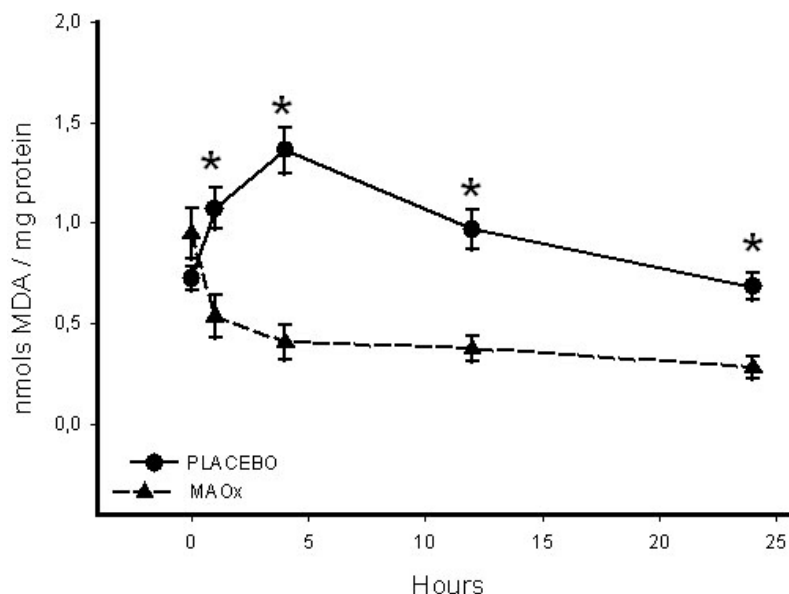
Figure 2. Malondialdehyde concentration measured at the different times of the study. Patient number was 16 for each one placebo and MAOx group, * p < 0.001.
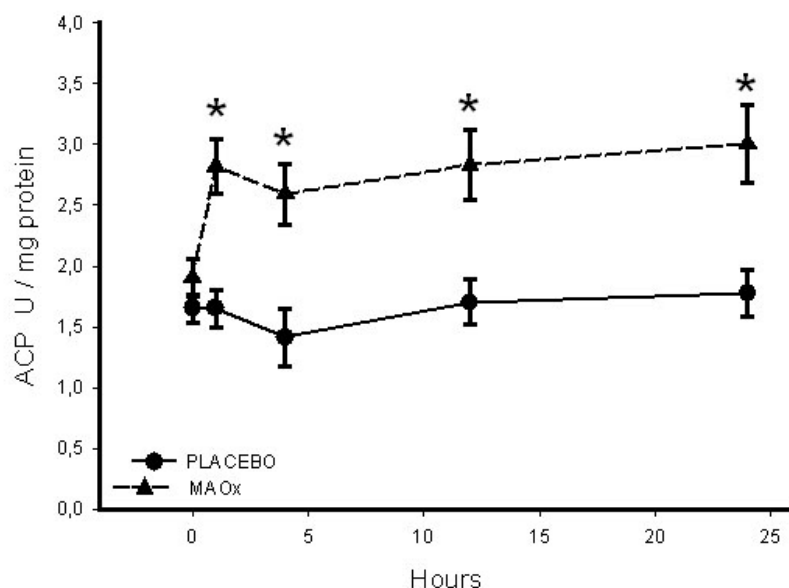

Figure 3. Antioxidant capacity of plasma expressed as Units. 1 U corresponds to the amount of plasma that inhibits 50 % of in vitro oxidation of a known mixture of substrates (linoleleic/linoleic acids 0.90 mmol/l) under controlled conditions of the Fenton reaction ($CuSO_4$ /H_2O_2, 4mmol/l and 5 mmol/l respectively). Patient number was 16 for each one placebo and MAOx group, * p < 0.01.

while significantly lower MDA concentrations were found in the MAOx group throughout the study period (Figure 2). In the MAOx group the MDA concentration decreased significantly (from 0.94 ± 0.42 to 0.53 ± 0.29 nmols MDA/mg protein) from 1 h after administration, with lowest values at 4 hr (0.40 ± 0.23 nmols MDA/mg protein).

The ACP values showed a significant increase in the MAOx group after 1 h of anti-oxidant treatment was initiated (from 1.89 ± 0.39 to 2.81 ± 0.86 Unit/mg protein) (Figure 3). This effect persisted throughout the duration of measurement (p<0.01), showing the highest value (3.00 ± 0.95 Unit/mg protein) 24 h after thrombolysis. The placebo group showed significantly lower ACP values than the MAOx patients throughout the study period. The placebo group showed no difference between time 0 (1.65 ± 0.36 Unit/mg protein) and 4, 12 or 24 h after thrombolysis (Figure 3).
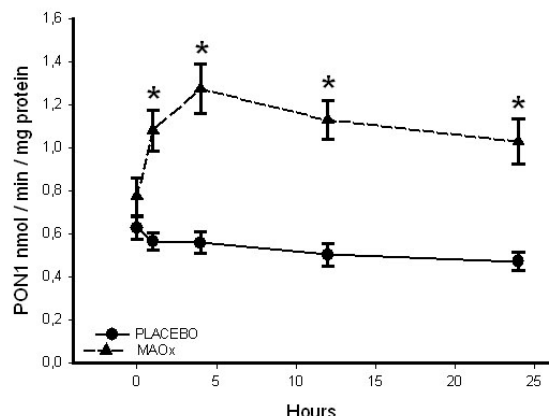
Figure 4. Paraoxonase activity. Patient number was 16 for each one placebo and MAOx group, * p < 0.001.

The PON activity increased from 1 h after MAOx administration (from 0.77 ± 0.08 to 1.08 ± 0.09 nmol/min/mg protein), peaking at 4 h (1.27 ± 0.11 nmol/min/mg protein) (Figure 4). The PON activities at 12 and 24 h were significantly higher than those in the placebo group. In the placebo group, there were showed no changes in the PON activity during the study period (0.62 ± 0.05 nmol/min/mg protein).

5. DISCUSSION

The biochemical and hemodynamic values measured during this study were similar in both groups and are characteristic of STEMI, showing typical increases of the biomarker enzymes CK, CK-MB,LDH, and AST at different times. However, analyzing and comparing the enzymatic parameters of tissue injury showed increases up to 12 h. In contrast, the plasma MDA concentration, which is considered as biomarker of lipoperoxidation induced by ROS (11, 25) increased only in the placebo group. The treated group showed no such increase and the MDA concentrations were similar to those present before thrombolysis. These findings show that treatment with MAOx does not prevent or decrease the level of injury to myocardial fibers. However, our data confirm and extend previous findings regarding the safety and benefits of combining an antioxidant (N-acetylcysteine) with nitroglycerin and streptokinase in the treatment of patients with evolving AMI (16). In the present study, we have demonstrated the efficacy of treatment with a mixture of antioxidant agents in decreasing the extent of injury due to oxidative stress (11, 18), which leads to complications after reperfusion therapy.

The most important clinical implications of the results reported here are related to the decrease of arrhythmias (premature ventricular beats, atrial fibrillation, ventricular tachycardia, first-degree atrial-ventricular block) in patients treated with MAOx. Despite the positive effect of MAOx, these anti-oxidants led to a significant increase in asymptomatic sinus bradycardia (from 49 to 59 bpm) in 50 % of the treated patients by an unknown mechanism, however this event did not require

pharmacological treatment, and had no effect on morbidity or mortality. The positive effect of MAOx is likely mediated by inhibition of the reactive oxygen species (ROS) which are generated after re-introduction of oxygen during reperfusion (3, 5). Sources for $O_2^{\bullet -}$ are vascular endothelium, neutrophils, fibroblasts, lymphocytes, phagocytes and platelets, which they get activated during STEMI (28). Platelets participate by utilizing the generation of ROS for intracellular signalling during activation, and recruit neutrophils. Neutrophils accumulate more slowly than platelets during reperfusion (29).

The decrease in arrhythmias by MAOx might be related to the scavenger capacity of MAOx for ROS (17), increasing the antioxidant capacity of the plasma (25) and significant decrease of circulating neutrophils (30). Another factor which might be contributing to the positive effect of MAOx is the increase in human serum paraoxonase 1 (PON1). PON1, a component of plasma HDL, plays an important role in protecting plasma lipoproteins and cell membranes from oxidative damage induced by ROS. PON1 effect might involve hydrolyzing specific lipid peroxides and thus interrupting the chain of lipoperoxidation of unsaturated fatty acids, with the consequent decrease of several oxidation products including MDA. The increase in PON1 in subjects treated with MAOx above that found in the control group persisted throughout the study. The increase in PON1reported here is consistent with those reported previously (31-32) including that increased by xenobiotic agents, antioxidants, and hormones (33-34).

In conclusion, the findings reported here suggest that once the factor(s) that underlie the increase in sinus bradycardia is identified and removed from the regimen used here, antioxidants might be useful as adjuvants in controlling reperfusion induced arrhythmias following therapeutic alteplase thrombolysis.

6. ACKNOWLEDGMENTS

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Abbreviations: INER: Instituto Nacional de Enfermedades Respiratorias, IMSS: Instituto Mexicano de Seguro Social, STEMI: ST- segment elevation myocardial infarction, IR: ischemia/reperfusion, ROS: reactive oxygen species, MAOx: mixture of antioxidant vitamins, MDA: malondialdehyde, ACP: antioxidant capacity of plasma, PON1: paraoxonase-1, RNS: reactive nitrogen species, AA: arachidonic acid, 20-HETE: 20-hydroxyeicosatetraenoic acid, NAC: N-acetylcysteine, EKG: electrocardiogram, CK-total: creatine kinase total, CK-MB: : creatine kinase MB-fraction, AST: aspartate aminotransferase, LDH: lactic dehydrogenase, TIMI: Thrombolysis In Myocardial Infarction, PVBs: single premature ventricular beats, VT: ventricular tachycardia, VF: ventricular fibrillation, AF: atrial fibrillation, SB: sinus bradycardia, FDAVB: first degree atrial-ventricular block, SN: sinus node, AV: atrial-ventricular node, SDAVB: second degree atrial-ventricular block, TDAVB: third-degree atrial ventricular block, BTH: butylated hydroxytoluene, TBA: thiobarbituric acid, AMI: acute myocardial infarction.

Key Words: Heart, Myocardium, Treatment, Anti-oxidant, Vitamins, Injury, Perfusion, Infarction, Reperfusion, Arrhythmias, Antioxidants, Malondialdehyde, Paraoxonase

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