Acute Myocardial Infarction Is Associated with Increased Susceptibility of Serum Lipids to Copper-Induced Peroxidation in Vitro

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Summary

Background: Low-density lipoprotein (LDL) oxidation in the arterial intima plays a pivotal role in atherogenesis. Under physiologic conditions, several mechanisms protect LDL against oxidation, including hydrolysis of oxidation products by high-density lipoprotein (HDL)-associated enzymes. Some of these protective mechanisms are less effective under acute phase conditions.

Hypothesis: Conditions of acute phase response, including acute myocardial infarction (MI), may be expected to result in increased susceptibility of serum lipids to oxidation. The present study was undertaken to test this possibility.

Methods: Using our previously developed spectroscopic method, we have monitored prospectively the kinetics of copper-induced oxidation of serum lipids obtained from 15 men during and after acute MI. This was tested within 6 h from the onset of chest pain, on Days 1, 3, and 7 of infarction and 1 year after recovery.

Results: The lag phase preceding oxidation of serum lipids was much shorter during the first week after MI when compared with values obtained after recovery (52–59 vs. 107 min, respectively, $p < 0.001$). During the first week after MI, we observed no significant correlations between kinetic parameters and serum lipid composition, in contrast both to the correlations previously reported for hyperlipidemic patients and to the similar correlations observed in the present study after recovery.

Conclusions: Acute MI is associated with an increased susceptibility of serum lipids to oxidation in vitro. This propensity for oxidation may reflect enhanced in vivo formation of free radicals and/or reduced efficiency of defense mechanisms. Both these possibilities may carry detrimental effects on the course, complications, and prognosis of the patients after acute MI.

Key words: myocardial infarction, lipid peroxidation, acute phase response

Introduction

Atherosclerosis is a complex, chronically developing disease caused by excessive accumulation of lipids (mostly cholesterol esters) in macrophages and smooth muscle cells, resulting in the formation of foam cells and in cell death.^{1,2} Oxidative modification of low-density lipoproteins (LDL) is believed to play a key role in this process.^{3–5} Oxidation of polyunsaturated fatty acids (PUFA) is followed by formation of breakdown products of lipid hydroperoxides, mostly aldehydes, which react with LDL-apolipoprotein B (apoB). The oxidatively modified LDL, no longer recognized by the LDL receptor, is ingested by macrophages via the scavenger receptor pathway, resulting in the formation of foam cells.^{3–6} In addition to its pivotal role in the atherogenic process, oxidized LDL is involved in destabilization of the atherosclerotic plaque and its rupture, in the thrombotic process, and in endothelial dysfunction.^{7–10}

Several lines of evidence^{11,12} indicate that under normal physiologic conditions high-density lipoprotein (HDL)-associated enzymes protect LDL from oxidation, thus reducing the risk of LDL oxidation in the arterial wall. This protective effect is compromised under conditions of acute phase.¹² Hence, acute phase response may be expected to result in increased susceptibility of plasma lipids to oxidation. Our present study was undertaken to test the possibility that acute myocardial infarction (MI), accompanied by an acute phase response, is associated with increased oxidizability of plasma lipids.

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Much effort has been devoted to the development of *in vitro* assays for measuring LDL oxidation. These include direct quantification of oxidation products in the circulation (oxidized LDL, hydroperoxides, TBARS) and measurement of LDL susceptibility *in vitro* to the addition of pro-oxidants such as copper ions.^{6,8} The results of the latter are expressed in terms of the "lag" preceding oxidation, which is commonly used as a measure of the resistance of lipids to oxidation. Because of potential experimental artifacts,¹³ mostly due to the need for LDL fractionation, the clinical usefulness of these assays is limited. To overcome this limitation we have previously developed a spectroscopic method that enables us to monitor the kinetics of copper-induced oxidation of serum lipids in unfractionated serum.¹⁴

Using this simple spectroscopic assay, we tested whether the acute phase response associated with acute MI is accompanied by a decrease in the resistance of serum lipids to copper-induced oxidation *in vitro*.

Methods

Subjects and Study Design

Fifteen male patients, aged 61.9 ± 12.1 years (38–74) [mean $\pm$ standard deviation (range)], admitted to Rabin Medical Center intensive coronary care unit (ICCU) (3–6 h after the onset of chest pain) for a first acute anterior MI and who survived the first year without revascularization procedures, were randomly included in this study. The diagnosis of acute MI was based on all three criteria: (1) typical chest pain, (2) ST-segment elevation $> 1 \text{ mm}$ $\pm$ the appearance of Q waves (wider than 0.04 s or deeper than 1/3 of R wave) in the anterior leads, and (3) a rise in plasma creatine phosphokinase (CPK) levels. With regard to cardiovascular risk factors, three patients were hypertensive, nine were smokers, nine were hyperlipidemic, and three were diabetic. After receiving informed consent, venous blood was drawn from each patient at five time points: (1) upon arrival at the ICCU, before receiving thrombolytic treatment (Day 0); (2) the morning after admission (Day 1); (3) on Day 3, (4) on Day 7 of hospitalization, and (5) approximately 1 year after hospitalization (202–493 days, mean 375 days). All samples except for those drawn on Day 0 were obtained after overnight fasting.

All but two patients underwent thrombolytic therapy with streptokinase. One patient who presented with syncope and another, aged 78, did not receive streptokinase; 12 patients were treated with heparin for 48 h.

At follow-up (after 1 year), a few patients reported having suffered from angina pectoris after discharge, although none suffered another infarction, unstable angina, or needed rehospitalization. At 1 year, the drugs consumed by our patients were as follows: statins—five patients; angiotensin-converting enzyme (ACE) inhibitors—four patients; calcium blockers—two patients; beta blockers—two patients; furosemide—three patients; amiodarone, warfarin, and low molecular weight heparin—one patient each.

Serum separated from all blood samples was frozen immediately at -70°C . Copper-induced oxidation of samples was performed after thawing at room temperature. All assays were performed simultaneously for any given patient. Each serum sample was analyzed also for triglycerides, total cholesterol, HDL-cholesterol, and CPK. White blood cell, platelet count, and fibrinogen were determined in parallel samples. Oral temperature was measured at each time point.

Copper-Induced Oxidation of Serum Lipids

Copper-induced oxidation was monitored at 37°C by continuous recording of absorbance at 245 and 268 nm, using a Uvikon 933 (Kontron Elektronik, Munich, Germany) double-beam spectrophotometer equipped with a 12-position automated sample changer. Measurements were carried out in quartz cuvettes (optical pathway 1 cm) after addition of CuCl_2 (final concentration 100 μM) and phosphate-buffered saline (NaCl 146 mM, NaH_2PO_4 3.3 mM, Na_2HPO_4 3.3 mM, pH = 7.4) to a solution containing 30 μl serum and 720 μM of sodium citrate (final volume 1.5 ml).¹⁴ The absorbance at 245 nm is attributed to dienic hydroperoxides and 7-ketocholesterol, whereas the absorbance at 268 nm is attributed mostly to dienals and other final products of decomposition of hydroperoxides.^{14,15} This method has been shown to correlate strongly with copper-mediated oxidation of LDL, as reported by Schnitzer *et al.*¹⁶

The kinetics of oxidation (Fig. 1) was analyzed in terms of (1) the maximal rate of accumulation of absorbing products ($V_{\max}$), (2) the time at which the rate of peroxidation achieved its maximal value ($t_{\max}$)—this parameter is in good correlation with the "lag" preceding oxidation and reflects the resistance of serum lipids to oxidative stress¹⁵—, and (3) the maximal accumulation of absorbing products ($\text{OD}_{\max}$).

Effect of freezing and thawing of samples: In order to allow simultaneous measurements of oxidation parameters for all time points of each patient, we studied the effect of freezing and thawing on these parameters. We analyzed fresh serum samples of nine patients and then repeated the analyses on the samples frozen immediately and thawed after 1 month. In agreement with our previous reports,^{14,16} we observed no significant effects on the measured parameters: coefficients of

FIG. 1 Kinetics of copper-induced oxidation of unfractionated serum ($V_{\max}$ and $t_{\max}$ were determined from the first derivative of the time course of absorption).

variation for $t_{\max}$ $4.69\% \pm 5.14\%$ ($p = \text{NS}$), for $V_{\max}$ $2.56\% \pm 3.95\%$ ($p = \text{NS}$), and for $\text{OD}_{\max}$ $5.24\% \pm 4.18\%$ ($p = \text{NS}$).

Heparin effect: The routine treatment protocol for patients with anterior MI at Rabin ICCU includes intravenous heparin therapy for 48 h, which might have affected the oxidation kinetics. To exclude this possibility, we monitored copper-induced oxidation of four different sera of nonheparin-treated patients before and after adding heparin *in vitro* to various concentrations exceeding therapeutic conditions: 0.85, 1.67, and 3.3 U/ml. Addition of heparin had no significant effect on the measured kinetic parameters: coefficients of variation for $t_{\max}$ $0.0\% \pm 5.9\%$ ($p = \text{NS}$), for $V_{\max}$ $2.66\% \pm 27.99\%$ ($p = \text{NS}$), and for $\text{OD}_{\max}$ $5.38\% \pm 29.08\%$ ($p = \text{NS}$).

Lipid Analysis

Triglyceride and cholesterol levels in the serum samples were measured by enzymatic commercial kits (Boehringer-Mannheim, Mannheim, Germany). The HDL cholesterol level was measured after precipitation of very low-density lipoprotein (VLDL) and LDL using heparin-MnCl₂. Estimation of LDL cholesterol level was made according to the Friedewald formula: LDL cholesterol = total cholesterol – HDL-cholesterol-TG/5.

Statistical Analysis

All data were analyzed by Microsoft Excel 7.0 (Microsoft Corporation, Seattle, Wash., USA) electronic spreadsheets.

Statistical analysis was performed using BMDP software (BMDP Statistical Software, Inc., Los Angeles, Calif.) and included means and standard deviations, correlations between oxidation parameters, and lipid profile and acute phase parameters. Variance of the measured parameters through time points after acute MI was performed by analysis of variance with repeated measurements for incomplete design. Multiple regression analysis was performed using Statistical Package for Social Sciences – 10.0 software (SPSS Inc., Chicago, Ill., USA). A p value of $< 5\%$ was considered significant.

Results

Upon admission to the ICCU (3–6 h after the onset of chest pain), the patients had elevated levels of CPK and white blood cells (WBC) (Table I). During the first week of hospitalization, the levels of CPK and inflammatory parameters (fever, WBC, fibrinogen, and platelets) changed as expected during acute MI.

Since the susceptibility of serum lipids to oxidation depends, among other factors, on the lipid composition in serum,¹⁵ we monitored the concentration of serum lipids during the first week of hospitalization and compared the results with those obtained after recovery. Compared with the postrecovery values, during the first week after MI, the levels of total cholesterol, LDL cholesterol, and triglycerides were 5–10% higher,

TABLE I Changes in studied parameters in 15 patients after myocardial infarction (mean $\pm$ SEM)

	3-6 h	Day 1	Day 3	Day 7	1 Year
Injury and acute phase					
CPK (I.U.)	1175 $\pm$ 389 ^a	2666 $\pm$ 728 ^a	327 $\pm$ 51 ^a	142 $\pm$ 42	116 $\pm$ 24
Fever (°C)	37.1 $\pm$ 0.7	37.6 $\pm$ 0.6 ^a	37.3 $\pm$ 0.6	36.8 $\pm$ 0.4	36.5 $\pm$ 0.2
WBC (X10 ³ /μl)	10.8 $\pm$ 1.2	12.8 $\pm$ 1.2	9.8 $\pm$ 1.1	8.8 $\pm$ 0.8	6.1 $\pm$ 0.4
Fibrinogen (mg/dl)	430 $\pm$ 55	344 $\pm$ 48	625 $\pm$ 72 ^a	732 $\pm$ 54 ^a	382 $\pm$ 24
Platelets (X10 ³ /μl)	206 $\pm$ 15	208 $\pm$ 23	171 $\pm$ 8 ^a	220 $\pm$ 29	222 $\pm$ 29
Serum lipids (mg/dl)					
TC	205 $\pm$ 14	198 $\pm$ 16	202 $\pm$ 12	200 $\pm$ 10	191 $\pm$ 8
LDL	136 $\pm$ 15	131 $\pm$ 15	130 $\pm$ 12	140 $\pm$ 9	124 $\pm$ 7
HDL	43 $\pm$ 3	44 $\pm$ 3	42 $\pm$ 3	31 $\pm$ 2 ^a	45 $\pm$ 2
TG	119 $\pm$ 18	114 $\pm$ 16	149 $\pm$ 17 ^a	154 $\pm$ 17 ^a	114 $\pm$ 12
Oxidation parameters 245 nm					
$t_{\max}$ (min)	56 $\pm$ 4 ^a	58 $\pm$ 6 ^a	59 $\pm$ 6 ^a	52 $\pm$ 5 ^a	107 $\pm$ 4
$V_{\max}$ (10 ³ xOD/min)	3.9 $\pm$ 0.4 ^a	3.4 $\pm$ 0.4 ^a	3.9 $\pm$ 0.4 ^a	3.8 $\pm$ 0.4 ^a	4.9 $\pm$ 0.3
$\text{OD}_{\max}$ (10 ³ xOD)	305 $\pm$ 24 ^a	256 $\pm$ 26 ^a	300 $\pm$ 29 ^a	257 $\pm$ 29 ^a	500 $\pm$ 38
$\text{OD}_{\text{initial}}$	1.55 $\pm$ 0.09	1.53 $\pm$ 0.07 ^a	1.58 $\pm$ 0.08 ^a	1.71 $\pm$ 0.07 ^a	1.38 $\pm$ 0.06
Oxidation parameters 268 nm					
$t_{\max}$ (min)	61 $\pm$ 7 ^a	58 $\pm$ 6 ^a	62 $\pm$ 6 ^a	52 $\pm$ 6 ^a	108 $\pm$ 5
$V_{\max}$ (10 ³ xOD/min)	2.7 $\pm$ 0.5 ^a	3.3 $\pm$ 0.6 ^a	3.2 $\pm$ 0.5 ^a	3.3 $\pm$ 0.5 ^a	3.9 $\pm$ 0.7
$\text{OD}_{\max}$ (10 ³ xOD)	245 $\pm$ 17 ^a	233 $\pm$ 13 ^a	259 $\pm$ 14 ^a	238 $\pm$ 14 ^a	297 $\pm$ 19

^a The current value of the parameter is significantly different from its value after 1 year ($p < 0.001$).

Abbreviations: SEM = standard error of mean, CPK = creatine phosphokinase, WBC = white blood cells, TC = total cholesterol, LDL = low-density lipoprotein, HDL = high-density lipoprotein, TG = triglycerides.

and HDL cholesterol was about 5% lower, although none of these changes reached statistical significance (Table I). During the first week of hospitalization, a transient elevation was observed in serum triglycerides (25–30%) ($p < 0.001$) and a 25% decrease in HDL levels ($p < 0.001$). Similar transient changes in lipid profile after MI have been observed previously.¹⁷

All kinetic parameters of oxidation measured during the first week after MI differed significantly from the respective values measured after recovery (Table I). Notably, none of these factors changed significantly within the first week. Representative kinetic profiles of serum peroxidation, as measured for one patient at three time points, are given in Figure 2. The most striking difference observed was during the first week: $t_{\max}$, which is commonly used as a measure of the resistance of lipids to oxidation, was half of its postrecovery value (52–59 vs. 107 min, $p < 0.001$). The kinetic results obtained at 268 nm were qualitatively similar to those obtained at 245 nm (Table I).

In agreement with our previous results,¹⁵ the kinetic parameters that characterize serum peroxidation observed in the present study after recovery (1 year) correlated with the lipid composition of the serum (Table II). Specifically, both $t_{\max}$ and $OD_{\max}$ correlated negatively with HDL; $V_{\max}$ correlated positively with total cholesterol and LDL; $OD_{\max}$ correlated positively with triglycerides. By contrast, during hospitalization (the acute phase), $t_{\max}$ exhibited no negative correlation with HDL. Notably, we observed no significant correlations between early or late values of serum lipid oxidizibility ($t_{\max}$) and the measured parameters of acute phase (including WBC, platelets, fibrinogen, or body temperature). Similarly, we observed no significant correlation between serum lipid oxidizibility and infarct size, as reflected by CPK levels (data not shown). In order to determine which of the measured variables are most associated with changes in serum lipid oxidizibility, we performed a multiple regression analysis: $t_{\max}$ (serum lipid oxidizibility) being the dependent variable, and smoking status, statin therapy, serum lipids and lipoprotein cholesterol, WBC, platelets, fibrinogen, fever, and CPK levels being the independent variables. None of these variables was found to be a statistically significant predictor of $t_{\max}$ ($p > 0.05$).

FIG. 2 Kinetics of copper-induced peroxidation of serum samples obtained from one patient at the designated times after myocardial infarction (recovery = 1 year).

Another interesting finding was that the initial OD level (at 245 nm) measured prior to addition of copper was significantly higher during acute MI than after recovery values (Table I).

Discussion

The major finding of the present study is that throughout the first week following MI the lipids in the serum were more susceptible to in vitro copper-induced peroxidation than after recovery (the lag preceding oxidation being much shorter). Moreover, during the acute phase the serum samples absorb more ultraviolet light (at 245 nm) than samples taken after recovery, probably reflecting higher concentrations of conjugated dienic hydroperoxides,^{5,18} formed via enhanced in vivo peroxidation of serum lipids.¹⁹ These results are consistent with the reported effects of acute phase response on oxidizibility of plasma lipids in patients undergoing cardiac surgery²⁰ or ischemic stroke.²¹ Our results also suggest that MI is associated with lowering of the oxidation resistance for at least 1 week after infarction, in agreement with previous data¹⁷ that showed that consequences of the acute phase may last up to 2 months. Notably, during the first week after the acute event, the correlations described by us previously between the composition of serum lipids and the oxidation parameters¹⁵ dissipate, probably because the impact of the acute phase proteins and oxidative radicals, which overrides the effects of lipid composition on oxidation kinetics. However, we observed no significant correlations between early or late values of serum lipid oxidizibility ($t_{\max}$) and the measured parameters of acute phase (including WBC, platelets, fibrinogen, or body temperature). Similarly, we observed no significant correlation between serum lipid oxidizibility and infarct size (as reflected by CPK levels).

TABLE II Correlations between kinetic parameters (245 nm) and lipid composition in sera of 15 patients after acute myocardial infarction

		Correlation coefficient for time (r)				
		3–6 h	Day 1	Day 3	Day 7	1 Year
$t_{\max}$	TG	–0.24	–0.15	–0.14	0.54 ^a	0.003
	TC	–0.1	0.24	–0.12	0.31	–0.38
	HDL	–0.35	–0.14	0.19	–0.45	–0.61 ^a
	LDL	0.1	0.32	–0.16	0.06	–0.26
$V_{\max}$	TG	–0.24	0.2	0.02	–0.18	0.35
	TC	0.56	0.82 ^b	0.65 ^a	0.31	0.66 ^b
	HDL	–0.22	0.47	0.23	0.43	–0.02
	LDL	0.7 ^a	0.77 ^b	0.59 ^a	0.3	0.65 ^b
$OD_{\max}$	TG	0.48	0.17	0.17	0.09	0.7 ^b
	TC	–0.35	0.74 ^b	0.36	0.21	0.4
	HDL	–0.76 ^a	0.07	0.3	0.23	–0.55 ^a
	LDL	–0.3	0.76 ^b	0.24	0.28	0.39

^a $p < 0.05$.

^b $p < 0.01$.

Abbreviations as in Table I.

The mechanism responsible for the effects of the acute phase response on lipid oxidizibility is not clear. One possibility is that HDL-associated proteins are involved. Specifically, acute phase response results in a decrease in both the level and activity of the antioxidative enzyme paraoxonase (as shown after MI)²² and increased level of the potentially pro-oxidative copper-chelator ceruloplasmin (as shown after cardiac surgery).²³ These changes may explain our results as well as those of previous studies showing that HDL taken from patients during the acute phase, unlike under normal conditions, did not protect LDL against oxidation induced by cocultures of artery wall cells.^{11, 12} Other acute phase reactants such as C-reactive protein (CRP) and serum amyloid A (SAA) protein, which interact with lipoproteins, may replace paraoxonase on the HDL particle, thereby enhancing the sensitivity of lipids to oxidation in vitro either by determining the level of hydroperoxides formed in vivo and/or by influencing the oxidation rate observed upon copper addition.¹² Unfortunately, we have not quantified serum CRP or SAA protein levels in our study.

Our study suffers several limitations. These include the putative effects of the change in diet, lifestyle, and medications, expected to occur after a major MI, on lipid levels and oxidation parameters. First, patients discharged from the ICCU are instructed to avoid saturated fats, which may affect lipid oxidizibility, in their diets. Second, oxidizibility may be affected by supplementary vitamins (E and/or C). Third, 9 of the 15 patients were smoking before the acute event and 6 of them reported to have stopped after hospitalization. This might also have contributed to the observed change in the oxidation parameters. Additional potential confounders were the effect of thrombolysis, heparin treatment, and freezing and thawing of samples.

We do not believe that any of the above changes can be held responsible for the large difference observed between the lag preceding oxidation during the post-MI hospitalization and that observed after recovery: (1) The dietary changes were presumably quite small, as indicated by the relatively minor changes in plasma lipid concentrations (Table I); (2) none of the patients consumed supplementary vitamins, neither before nor after acute MI; (3) the lag preceding oxidation did not change significantly during the 7 days of hospitalization, and since the patients did not smoke during this period it appears that smoking cannot be regarded to be a major cause of the apparent effect of MI on lipid oxidizibility. Moreover, the smoking status was not found to affect oxidation parameters by multivariate analysis; (4) most medications have been added during hospitalization, so that most of their effects should have been observed during hospitalization; specifically, statin therapy was found to carry no effect on $t_{\max}$ by multivariate analysis; (5) thrombolysis cannot be considered to be a major confounder, as none of the oxidation parameters differed significantly between admission (before thrombolysis) and repeated measurements during hospitalization (after thrombolysis); (6) the putative effect of heparin has been excluded (see Methods) by analysis of the effect of adding heparin to serum samples in vitro. Furthermore, the values of the measured oxidation parameters did not differ significantly between admission (be-

fore heparin therapy) and the repeated measurements during hospitalization (during heparin treatment), indicating that heparin treatment cannot explain the differences observed in oxidation parameters at the different time points after MI; (7) the possibility that freezing and thawing of serum samples had a significant effect on the reported results has been excluded by comparing the kinetic parameters of oxidation on paired samples, frozen and unfrozen^{14, 16} (Methods).

Based on these considerations, although we have no knowledge of the kinetics of oxidation before the acute event, we propose that the increased oxidizibility of serum lipids observed in patients with MI may be due to the acute phase response. Yet, this high oxidative stress might have been present prior to MI and might have triggered it.²⁴ As the postrecovery oxidation parameters in our patients are similar to those reported previously¹⁵ for hyperlipidemic outpatients, it may be assumed that the observed postrecovery values represent pre-infarction values. Hence, it is conceivable that the acute event is associated with a decrease in the resistance to serum lipid peroxidation.

Conclusions

Under conditions of our assay, patients suffering acute MI show increased oxidizibility of serum lipids. Regardless of the actual mechanism responsible for this increased susceptibility to oxidation, it may be concluded that patients with MI are subject to high oxidative stress. In the context of the conflicting results regarding the role of antioxidant therapy in atherosclerosis,^{25–29} it has been speculated that patients subject to high oxidative stress are likely to benefit from antioxidant supplementation. This notion is in agreement with the results of three recent studies that demonstrated significant beneficial effect of antioxidants in animals with myocardial injury,²⁵ in humans after angioplasty,²⁶ and in patients on hemodialysis.²⁹ Hence, antioxidants may improve the prognosis of patients with MI, particularly because products of oxidation may accelerate not only the atherogenic process but also its late and often fatal consequences of plaque rupture, thrombosis, and endothelial dysfunction.^{7–10} Therefore, patients under conditions characterized by acute phase response may also benefit from antioxidant treatment. These possibilities, however, still await further clinical studies.

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