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HIGH-DOSE STATIN PRIOR TO PRIMARY PERCUTANEOUS CORONARY INTERVENTION REDUCES OXIDATIVE STRESS BURDEN IN PATIENTS WITH ACUTE ST-ELEVATION MYOCARDIAL INFARCTION

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ABSTRACT. The current study analysed the effect of high-dose statin loading prior to primary percutaneous coronary intervention on oxidative stress markers, in patients with acute ST-elevation myocardial infarction (STEMI). Besides the lipid lowering effect, statins have antioxidant properties that might reduce myocardial ischemia-reperfusion injury. From a total of 37 patients, 18 patients received high-dose statin before coronarography and were included in the statin group, while 19 statin naive patients were included in the control group. Peripheral venous blood samples were obtained before coronary reperfusion, at 1 hour and 24 hours after that. The following markers of oxidative stress were determined from the serum: malondialdehyde (MDA), reduced glutathione to oxidized glutathione ratio (GSH/GSSG) and total antioxidant capacity (TAC). Values are shown as medians and interquartile ranges. MDA concentration and TAC had non-significant differences between the two groups, at all time frames. Before angioplasty, GSH/GSSG ratio was comparable between the two groups: 3.59 (2.13-5.37) in the statin group vs 2.69 (2.15-5.02) in the control group, p=0.49. At 1 hour after reperfusion, values were still similar: 2.26 (1.32-4.28) in the statin group vs 2.33 (1.88-2.50) in the control group, p=0.55. After 24 hours, there was a significant increase of GSH/GSSG ratio in the statin group 2.41 (1.58-3.28) vs 1.56 (1.12-2.03) in the control group, p=0.01. This finding suggest that, in STEMI patients, high-dose statin loading before primary percutaneous coronary intervention significantly reduces oxidative stress burden, early after administration.

Keywords: *oxidative stress, acute myocardial infarction, statin, glutathione, percutaneous coronary intervention*

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INTRODUCTION

Cardiovascular diseases are the leading cause of mortality and morbidity worldwide [1]. Acute ST-elevation myocardial infarction (STEMI) is associated with total coronary occlusion and subsequent myocardial necrosis. The principal objective of therapy is rapid restoration of coronary blood flow to save as much heart muscle as possible. According to current evidence-based guidelines, primary percutaneous coronary intervention (pPCI) is the best method of vessel opening [2,3]. However, a sudden restoration of blood supply, to a previously ischemic myocardium, can lead to myocardial reperfusion injury [4] and increased oxidative stress [5-7] that can paradoxically reduce the beneficial effect of angioplasty.

Statins are lipid-lowering drugs that inhibit cholesterol biosynthesis via down-regulation of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. Large secondary prevention trials showed that statins lower the rate of myocardial infarction, ischemic stroke and cardiovascular death [8]. The favourable effect on cardiovascular events depends mostly on the cholesterol-lowering function and plaque stabilization [9], but statins have important pleiotropic effects, like increased nitric oxide production, reduced oxidative stress generation and down-regulation of proinflammatory biomarkers [9]. Overall, statins can have an antioxidant effect, early after administration [10].

The ratio of reduced glutathione (GSH) to oxidized glutathione (GSSG) is an indicator of cellular health, with GSH constituting up to 98% of total glutathione under normal conditions. In various models of oxidative stress, this ratio has been demonstrated to decrease to values of 1:1, meaning that the lower the ratio, the more intense the oxidative process is [11]. In experimental models, measuring GSH/GSSG ratio is an excellent way to assess potential therapeutics efficacy in maintaining cellular redox potential [12].

Reactive oxygen species degrade polyunsaturated lipids, forming malondialdehyde (MDA). This stable end-product aldehyde is used as a biomarker to measure the level of oxidative stress in an organism [13-15].

Total antioxidant capacity (TAC) is a measurement used to assess the antioxidant status of biological samples and can evaluate the antioxidant response against the free radicals produced in a given disease [16-18].

Among patients with STEMI, the current guidelines recommend routine administration of high-dose statin [2,3]. However, the ideal timing of statin initiation, in the acute setting, is not stated. Recent data showed that, in STEMI patients, high-dose statin administration before pPCI significantly reduced 30-day major adverse cardiac events (MACE) [9]. The mechanisms leading to the benefit remain uncertain.

The aim of the current study was to determine the influence of high-dose statin loading prior to pPCI on oxidative stress markers (MDA, GSH/GSSG ratio and TAC), in STEMI patients. The variation of oxidative stress markers was statistically evaluated using the Mann-Whitney U test for non-normally distributed variables.

RESULTS AND DISCUSSION

Thirty-seven patients were included in the study, 18 patients in the statin group and 19 patients in the control group. The baseline demographic and clinical characteristics are presented in Table 1. The measured variables of the two groups are shown in Table 2.

Table 1. Baseline characteristics of the study groups (SD = standard deviation, TSR = time from symptom-onset to reperfusion, BMI = body-mass index)

STATIN PRELOAD	**YES**	**NO**
Patients, no (%)	**18 (48.6)**	**19 (51.4)**
Presentation		
Age (years), mean ± SD	66 ± 9.5	60 ± 12
Sex (male), no (%)	10 (55.5)	14 (73.6)
TSR (hours), mean ± SD	7.3 ± 4	5.4 ± 3
Risk factors		
Hypertension (yes), no (%)	13 (72.2)	13 (68.4)
Dyslipidemia (yes), no (%)	13 (72.2)	11 (57.8)
Smokers (yes), no (%)	6 (33.3)	8 (42.1)
Diabetes (yes), no (%)	5 (27.8)	2 (10.5)
BMI (kg/m2), mean ± SD	28.9 ± 4.8	29.2 ± 3.9
Type of statin		
Atorvastatin 80 mg, no (%)	13 (72.2)	
Atorvastatin 40 mg, no (%)	2 (10.5)	
Simvastatin 40 mg, no (%)	2 (10.5)	
Rosuvastatin 20 mg, no (%)	1 (6.8)	

Table 2. Variables of the study groups according to the presence of statin (MDA = malondialdehyde (nmol/ml), GSH/GSSG = reduced glutathione / oxidised glutathione ratio, TAC = total anitioxidant capacity (inhibition %), P_0 = before reperfusion, P_1 = 1 hour after reperfusion, P_{24} = 24 hours after reperfusion)

	MDA			GSH/GSSG			TAC		
With statin	**P_0**	**P_1**	**P_{24}**	**P_0**	**P_1**	**P_{24}**	**P_0**	**P_1**	**P_{24}**
1	2.82	3.11	2.49	5.44	1.73	2.38	24.33	24.20	24.07
2	2.03	1.65	0.76	8.35	4.25	3.98	38.48	41.28	42.25
3	2.91	2.61	2.08	4.41	0.95	1.59	36.40	33.44	32.21
4	2.01	1.84	1.66	6.56	5.34	2.56	32.38	33.95	37.61
5	4.71	3.87	4.23	3.10	1.21	1.43	27.06	26.32	28.41
6	2.69	2.49	2.20	4.55	0.90	1.82	27.03	26.82	21.26
7	2.95	2.58	1.31	2.94	1.52	2.43	36.14	41.62	37.45
8	4.06	2.57	2.03	1.98	1.99	2.02	29.57	34.13	31.53
9	2.69	2.40	1.37	5.35	4.35	3.25	39.81	37.85	37.42
10	4.01	3.43	3.97	3.36	2.65	1.98	32.46	37.02	36.56
11	3.28	3.06	2.70	2.18	1.98	5.66	32.97	31.57	31.10
12	2.80	2.08	1.93	1.48	3.99	3.26	34.3	33.45	30.52
13	3.30	3.27	3.03	1.46	1.15	1.31	39.88	30.18	29.32
14	1.59	1.64	2.22	8.38	4.88	4.95	37.63	40.25	39.71
15	2.19	1.17	0.75	3.81	4.06	3.05	26.19	27.85	28.98
16	3.16	2.28	1.32	1.25	1.35	1.05	28.38	28.02	25.72
17	3.44	2.44	2.81	2.97	8.10	3.34	27.91	20.05	22.40
18	4.16	3.19	2.33	4.25	2.53	1.53	36.45	31.54	41.67
Without statin									
1	2.46	1.88	1.69	1.35	1.54	1.12	36.07	37.61	33.41
2	3.56	3.35	1.66	5.63	2.35	3.25	25.44	25.24	20.39
3	4.13	3.02	2.66	9.60	2.44	2.62	37.22	36.03	33.11
4	1.94	2.09	1.66	7.96	2.33	1.56	37.61	26.03	26.66
5	4.02	3.01	3.77	5.02	2.98	0.79	29.09	32.90	35.61
6	3.48	3.20	1.54	2.31	1.88	1.79	39.13	37.49	41.74
7	4.71	3.57	2.43	1.35	1.05	0.53	33.35	29.01	32.25
8	1.96	1.92	1.21	2.63	2.02	1.25	32.88	31.48	32.64
9	3.96	3.66	2.43	3.57	3.02	3.12	32.21	36.13	41.38
10	4.53	3.80	3.34	2.25	2.38	1.36	29.64	31.05	31.63
11	1.96	1.72	1.06	1.65	1.36	1.98	32.38	31.45	36.39
12	2.86	2.71	1.62	2.15	1.88	1.02	35.65	37.72	35.70
13	3.02	3.67	3.94	2.69	1.04	1.15	41.86	49.15	58.22
14	3.45	4.35	2.83	3.58	2.53	2.65	28.84	18.48	12.80
15	3.30	2.17	0.53	6.35	2.34	1.25	31.26	31.68	41.93
16	2.86	2.46	2.22	2.32	2.62	1.96	24.15	22.09	21.81
17	4.98	3.86	3.15	3.25	2.04	1.96	25.96	25.50	27.68
18	1.28	1.71	1.09	3.25	2.50	2.03	32.64	31.08	37.64
19	1.48	1.29	1.52	1.65	2.02	1.02	37.06	37.38	43.41

GSH/GSSG ratio before pPCI (P_0) was comparable between the two groups: 3.59 (2.13-5.37) in the statin group vs 2.69 (2.15-5.02) in the control group, p=0.49. At 1 hour after pPCI (P_1), no significant differences were noted: 2.26 (1.32-4.28) in the statin group vs 2.33 (1.88-2.50) in the control group, p=0.55. After 24 hours (P_{24}), there was a significant increase in GSH/GSSG ratio in statin loaded patients: 2.41 (1.58-3.28) in the statin group vs 1.56 (1.12-2.03) in the control group, p=0.01 (Figure 1).

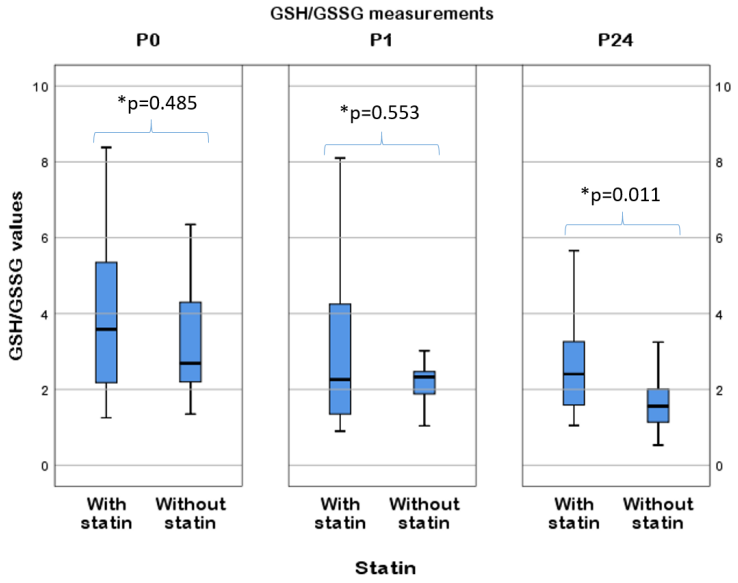

Figure 1. Comparison of GSH/GSSG values for P_0, P_1 and P_{24} measurements according to the presence of statin (* Mann-Whitney U Test; bars = range, box = first quartile to third quartile, horizontal black line = median)

At P_0, the median MDA levels were 2.93 (2.57-3.58) nmol/ml for the statin group and 3.3 (1.96-4.02) nmol/ml in the control group, p=0.68. There were no differences between the two groups at 1 hour (P_1): 2.53 (2.02-3.12) nmol/ml in the statin group vs 3.01 (1.92-3.66) nmol/ml in the control group, p=0.34. Also at P_{24}, MDA levels were similar: 2.14 (1.36-2.73) nmol/ml in the statin group vs 1.69 (1.52-2.83) nmol/ml in the control group, p=0.84 (Figure 2).

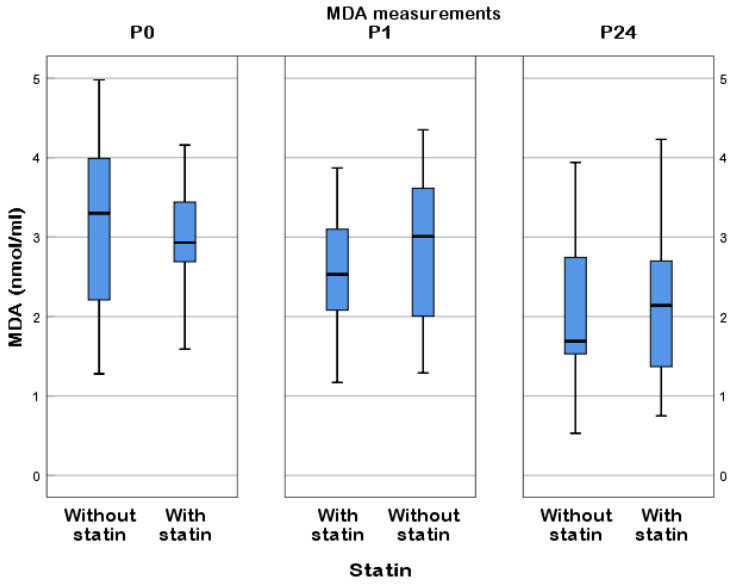

Figure 2. Comparison of MDA values for P_0, P_1 and P_{24} measurements according to the presence of statin (bars = range, box = first quartile to third quartile, horizontal black line = median)

TAC levels were not significantly influenced by statin loading. Baseline levels (P_0) were similar: 32.72 (27.69-36.75) inhibition % in the statin group vs 32.64 (29.09-37.06) inhibition % in the control group, p=0.94). Furthermore, after pPCI no significant changes were noted: P_1, 32.51 (27.59-37.21) inhibition % in the statin group vs 31.48 (26.03-37.38) inhibition % in the control group, p=0.73; P_{24}, 31.32 (27.74-37.49) inhibition % in the statin group vs 33.41 (27.68-41.38) inhibition % in the control group, p=0.44 (Figure 3).

In the settings of acute myocardial infarction, previous papers showed that after vessel opening there is an increase in oxidative stress burden from the first minutes and lasting several days [5,14,19]. These studies showed that some oxidative stress parameters, such as MDA are improved quickly after reperfusion [5,14,19], but TAC [14] and GSH/GSSG ratio [5] decreased after reperfusion, suggesting myocardial reperfusion injury.

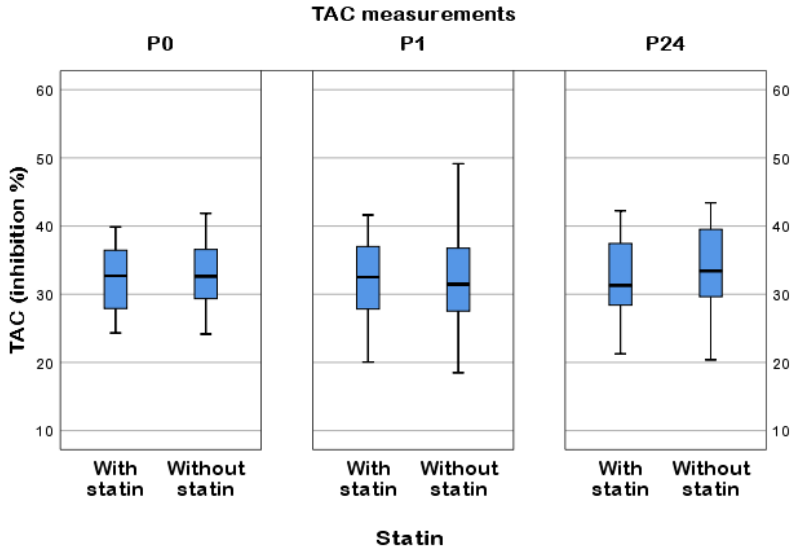

Figure 3. Comparison of TAC values for P_0, P_1 and P_{24} measurements according to the presence of statin (bars = range, box = first quartile to third quartile, horizontal black line = median).

The main finding of the present study showed that, in STEMI patients, high-dose statin loading before pPCI significantly reduced oxidative stress burden by improving GSH/GSSG ratio at 24 hours after successful reperfusion. An improved GSH/GSSG ratio can explain the benefit of statin loading prior to pPCI, by protecting against myocardial reperfusion injury.

Considering that the reduction in oxidative stress occurred early, the mechanism behind this potential effect is probably not the low-density lipoprotein cholesterol reduction. Two major trials [9,20] also support the hypothesis that the possible benefit of statin therapy could extend beyond a lipid-lowering effect.

The CANTOS trial [20] showed that anti-inflammatory intervention reduces MACE in patients with coronary artery disease. Considering that pPCI may result in both local and embolic complications, enhancement of inflammatory activity and atherosclerotic plaque instability [21], these additional effects of statins have the potential to reduce the risk of clinical events.

As mentioned above, a subgroup analysis of the SECURE-PCI trial [9], showed that STEMI patients undergoing pPCI that had a loading dose of 80 mg atorvastatin before angiography had a significant reduction in MACE at 30 days: hazard ratio, 0.54; 95% confidence interval, 0.35-0.84; p=0.01.

MDA results as a product of polyunsaturated fatty acid degradation [13]. MDA levels may be linked to the lipid lowering property of statins, so it is not likely that MDA can decrease in a few hours after statin administration. Two papers, that included patients at high-risk of cardiovascular disease, showed a significant reduction in MDA levels after 4 to 12 weeks of statin treatment [22,23]. In contrast, a recent paper with a similar study population, failed to show a significant reduction in MDA levels in patients treated with atorvastatin or simvastatin [24]. In the positive studies, lipid peroxidation was evaluated by the thiobarbituric acid (TBA) fluorescence method, which has potential limitations with respect to sensibility and specificity. The negative study, used the high-performance liquid chromatography (HPLC) method, which is more specific [25-28].

The potential of statin therapy to increase TAC was demonstrated in patients with established coronary artery disease [29], after a 3-month period of treatment. There are different methods for determining TAC. The scavenging of 1,1-diphenyl-2-picrylhydrazyl (DPPH) was not sensible enough for the acute settings [5], but the quenching of the (2,2'-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid)) radical cation by antioxidants was performed in another pPCI based study, with promising results [14].

So far, in patients with STEMI, early administration of statins before pPCI is not recommended in evidence-based guidelines, but increasing evidence of benefit may guide medical decision making, in these critical scenarios.

CONCLUSIONS

In STEMI patients, high-dose statin loading before pPCI significantly reduced reperfusion induced oxidative stress, as reflected by the increase in GSH/GSSG ratio at 24 hours after angioplasty.

Statin loading did not influence MDA and TAC levels.

EXPERIMENTAL SECTION

The study was in accordance with the Declaration of Helsinki [30]. Written consent was obtained from each patient before the procedure. Patients diagnosed with acute STEMI and admitted to Cluj County Emergency Hospital, Interventional Cardiology Department, were enrolled between April 2017 and December 2017. Inclusion criteria were as follows: electrocardiographic evidence of ST elevation of ≥ 1 mV in two or more standard limb leads or

≥ 2 mV in two or more precordial leads; typical chest pain lasting more than 20 minutes; time of presentation under 12 hours since symptom onset. All patients received drug eluting stents and pPCI was confined to the infarct-related artery. Only patients with successful reperfusion were included in the study. All patients were pretreated with antithrombotic medication according to current recommendations [2,3,31]. All demographic, clinical, paraclinical and intraprocedural data were stored in the Departments' structured database for future comparing [32,33].

Patients which received high-dose statin in the Emergency Room, before transferring to the Catheterization Laboratory, were included in the statin group. Patients that did not receive statin before pPCI and were not on chronic statin therapy (statin naive) were included in the control group. All patients subsequently received statin therapy 24 hours after pPCI. High-dose statin treatment was defined as follows: atorvastatin 40 mg or 80 mg, rosuvastatin 20 mg or 40 mg and simvastatin 40 mg or 80 mg.

Peripheral venous blood samples were obtained from each patient immediately before pPCI (P_0) and then after 1 hour (P_1) and 24 hours (P_{24}). Samples were drawn into plastic tubes with ethylenediaminetetraacetic acid (EDTA). Blood was centrifuged within 1 hour at 1500 rpm for 15 minutes and the collected plasma was stored at –30° C until analysis (within 1 month).

All the reagents were supplied by Sigma (Deisenhofen, Germany), were of analytical grade and were used without further purification.

MDA was determined using the method of Conti [34]. Through this process, the resulting MDA reacts with TBA to form a fluorescent adduct. First, 50 µl of plasma was boiled with 1 ml of 10 mM 2-TBA and 1 ml of 75 mM K_2HPO_4, pH=3. After quencing, the product was extracted with n-butanol. The fluorescence of the extract was measured at an emission wavelength of 534 nm using a spectrofluorometer (Lambda 35, Perkin Elmer, USA) with a synchronous fluorescence technique at a difference of 14 nm between the excitation and emission wavelength ($\Delta\lambda$). MDA concentration was determined based on a calibration curve consisting of common MDA concentrations using the same measurement technique. MDA levels are expressed in nmol/ml.

GSH was determined as described by Hu [35]. First, 500 µl of plasma are mixed with 500 µl of cold 10% trichloroacetic acid (TCA). After 10 minutes in ice, the mixture is centrifuged at 3000 rpm for 15 minutes, and then, 200 µl of the supernatant is mixed with 1.7 ml of phosphate buffer and 0.1 ml of o-phthalaldehyde. After 15 minutes, the fluorescence at 350 nm excitation and 420 nm emission is read against a blank (Lambda 35, Perkin Elmer, USA).

GSSG was calculated using Vats' method [36]. Initially, 250 µl of the plasma sample was incubated with 0.1 ml of 40 nM N-ethylmaleimide for 30 minutes, followed by addition of 0.65 ml of 0.1 M NaOH. Thereafter, the same procedure was followed for fluorescence development as used in

GSH measurement, except in place of the buffer, 0.1 M NaOH was used. The amounts of GSH and GSSG were calculated from standard curves and are expressed in µmol/ml.

TAC was determined according to Janaszewska [37].The reduction assay was performed by adding 20 µl of plasma to 400 µl of 0.1 mM methanol solution of DPPH and phosphate buffer, pH=7.4. After a 30-minute incubation at ambient temperature, absorbance of the samples at 520 nm was measured (Lambda 35, Perkin Elmer, USA) and compared with that of a reference sample containing only DPPH solution and phosphate buffer. TAC was measured in inhibition % as [(control extinction - serum extinction) / control extinction] x 100.

The statistical analysis was conducted using SPSS software v25 (IBM, USA). The Shapiro-Wilk test was used to assess for a normal distribution. Quantitative data without normal distribution was described using box-plots, median and Q1-Q3, where Q1-Q3 (inter quartile range) stands for the range between 25th percentile (Q1) and 75th percentile (Q3). Normally distributed data was presented as mean ± standard deviation. The Mann-Whitney U test was applied for non-normally distributed variables to check if there was a significant difference between the two independent samples. A p-value equal to or lower than 0.05 was considered statistically significant.

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