RESEARCH LETTER

Oxidative stress markers, thioredoxin 1 and 8-isoprostane, in relation to ischemic time in patients with ST-segment elevation myocardial infarction treated by primary percutaneous coronary intervention

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Introduction The extent of ischemia-reperfusion injury (IRI) significantly affects the prognosis of patients with ST-segment elevation myocardial infarction (STEMI). Excessive generation of reactive oxygen species during ischemia and subsequent reperfusion leads to cellular necrosis and apoptosis. These processes contribute to the impairment of microcirculation and the no-reflow phenomenon, development and progression of left ventricular remodeling and failure.[1]

Out of numerous factors affecting the complex process of IRI, the duration of ischemia is of major importance. Prolonged ischemia has been associated with higher degree of oxidative stress, but only scarce evidence is available up to date.[2]

Our goal was to evaluate selected markers of oxidative stress in relation to reperfusion via primary percutaneous coronary intervention (pPCI) and their potential correlation with the duration of ischemia, defined as time delay between symptom onset and reperfusion.

Patients and methods **Study population** A total of 60 patients (mean age, 64.9 years; 24.4% women) were prospectively included in the study: 45 had acute STEMI and underwent pPCI and 15 controls underwent diagnostic coronary angiography (CAG). Definition of STEMI was based on the criteria established by the European Society of Cardiology. The inclusion criteria for patients with STEMI included 1-vessel disease and known time of symptom onset. We subsequently excluded patients with preprocedural Thrombolysis in Myocardial Infarction risk score of more than 1 or postprocedural score of less than 2; thus, 30 patients and 15 controls were included for further analysis (TABLE 1). The patient selection process is shown in Supplementary material, *Figure S1*. The control group included individuals undergoing CAG with no significant lesions in coronary arteries. For exclusion criteria, see Supplementary material.

Time delay to treatment was defined as the interval from symptom onset as stated by the patient to the first balloon inflation. Out of the followed in-hospital complications (stent thrombosis, cardiac and overall mortality, heart failure, new-onset atrial fibrillation, ventricular tachycardia, or ventricular fibrillation), only new-onset atrial fibrillation occurred during hospitalization (TABLE 1).

The institutional review board at University Hospital Kralovske Vinohrady, Prague, Czech Republic approved the study and all patients gave written informed consent for the participation.

Laboratory assessment The venous blood samples were collected immediately before the pPCI, then 1 hour, 6 hours, and 48 hours after pPCI. In controls, the first blood sample was drawn at the first catheter insertion at CAG and the following blood samples were collected at the identical

TABLE 1 Baseline characteristics of the study population

Parameter	Patients (n = 30)	Controls (n = 15)	*P* value
Age, y, mean (SD)	64.7 (13.6)	65.3 (11.2)	0.88
Female sex, n (%)	8 (26.7)	3 (20)	0.73
BMI, kg/m^2, mean (SD)	27.7 (5.3)	28.9 (5.4)	0.48
BSA, m^2, mean (SD)	1.97 (0.26)	1.95 (0.18)	0.78
Time delay to reperfusion, h, median (IQR)	4.32 (2.64–9.84)	–	NA
Hypertension, n (%)	20 (67)	9 (60)	0.55
Diabetes mellitus, n (%)	8 (18)	4 (27)	0.41
Smoker, n (%)	19 (63)	9 (60)	0.72
Dyslipidemia, n (%)	5 (17)	3 (20)	1.0
Creatinine clearance, ml/min, mean (SD)	70.5 (24)	53.3 (20.2)	0.057
LVEF, mean (SD)	43.6 (9.8)	56.3 (7.9)	<0.001
Culprit lesion, n (%)			
LAD	16 (53.3)	–	NA
RCA	14 (46.7)	–	NA
Killip class, n (%)			
1	23 (82.1)	–	NA
2	3 (10.1)	–	NA
3	1 (3.6)	–	NA
4	1 (3.6)	–	NA
In-hospital complications, n (%)			
New-onset AF	5 (11.1)	–	NA
In-hospital mortality	0	–	NA

Abbreviations: AF, atrial fibrillation; BMI, body mass index; BSA, body surface area; IQR, interquartile range; LVEF, left ventricular ejection fraction; LAD, left anterior descending coronary artery; NA, not applicable; RCA, right coronary artery

time intervals as in patients with STEMI. Blood was allowed to clot for 30 minutes after the collection and centrifuged (2500 G, 15 minutes) afterwards. Serum was collected from the samples and stored in an anonymized manner at –80 °C until analyzed.

Because of the high instability of reactive oxygen species and complicated direct measurement, intensity of IRI can be assessed indirectly by evaluating stable downstream products of the oxidative damage.[1] We chose to assess a broader spectrum of oxidative stress markers to cover different types of cellular damage, including 8-isoprostane (8-ISO), a lipid peroxidation marker produced from arachidonic acid via a free radical–catalyzed mechanism;[3] superoxide dismutase 3 (SOD3), a major enzymatic antioxidant defense against superoxide radical;[4] 8-hydroxy-2′-deoxyguanosine, a product of oxidative DNA damage;[5] cytochrome c, a mitochondrial protein, released into the cytosol following reperfusion;[6] 3-nitrotyrosine, produced by the modification of tyrosine residues in proteins by peroxynitrite;[7] and thioredoxin-1 (TRX1), a redox-active protein induced by oxidative stress.[8]

Laboratory methods are described in the Supplementary material, the *Laboratory analysis* section.

Statistical analysis Exploratory data analysis was performed for all parameters. Continuous parameters are expressed as mean (SD) or median (interquartile range) according to data distribution, and categorical parameters as count and frequency. Baseline characteristics were compared using the *t* test and the Fisher exact test or χ^2 test.

Parameters with log-normal distribution (marker levels) were logarithmically transformed for further analysis (decimal logarithm), and the Shapiro–Wilk test was used to examine the agreement of the distribution of variables with log-normal distribution.

Temporal changes in biomarker levels (visit 1–4) were analyzed using a linear mixed-effects model with factorial design, random intercept, and maximum likelihood estimator. Time delay cutoff values of 3, 6, and 9 hours after pain onset were tested using the *t* test to assess the differences between groups below and above the cutoff values.

Post hoc analysis was adjusted for multiple comparison using the Tukey test. The analyses were adjusted for possible confounding factors (left ventricular ejection fraction, creatinine clearance). The results of statistical analyses are presented as geometric mean (95% CI). All statistical tests were evaluated at a significance level of 0.05.

Results Out of all evaluated markers, the pre-pPCI levels of 8-ISO were higher in patients with STEMI compared with controls (geometric mean [95% CI], 68.83 [42.29–112.02] pg/ml vs 22.58 [10.95–46.57] pg/ml; $P = 0.027$). The difference among patients and controls persisted 1 hour after pPCI/CAG (geometric mean [95% CI], 76.35 [46.91–124.26] pg/ml vs 27.75 [13.34–57.69] pg/ml; $P = 0.045$). At 6 hours after pPCI, 8-ISO levels decreased compared with baseline values (geometric mean [95% CI], 30.91 [18.99–50.30] pg/ml; $P < 0.001$) in patients with STEMI. No changes of 8-ISO with time were observed in controls.

TRX1 levels tended to be higher in patients 1 hour after pPCI compared with controls at a corresponding time point (geometric mean [95% CI], 5.73 [4.05–8.11] ng/ml vs 3.35 [2.01–5.59] ng/ml; $P = 0.11$); however, the difference did not reach statistical significance. No periprocedural changes in marker levels were observed in controls. Serum levels of TRX1 and 8-ISO in relation to pPCI / CAG are presented in Supplementary material, *Figures S2* and *S3*.

We did not observe any significant periprocedural changes in the levels of 8-hydroxy-2′-deoxyguanosine, cytochrome c, SOD3, and 3-nitrotyrosine among patients and controls.

The median (interquartile range) time delay to pPCI was 4.32 (2.64–9.84) hours.

The pre-pPCI TRX1 levels were higher in patients with time delay to pPCI of 6 hours or longer when compared with patients with time delay of less than 6 hours (12.41 [6.78–22.72] ng/ml vs

5.57 [3.47–8.93] ng/ml; $P = 0.048$) (Supplementary material, *Figure S4*).

The levels of 8-ISO in patients with time delay to pPCI of 3 hours or longer tended to be higher than in patients with time delay of less than 3 hours at 1 hour after pPCI, but the difference did not reach statistical significance (81.11 [45.17–145.65] vs 31.36 [13.11–75.06]; $P = 0.09$) (Supplementary material, *Figure S5*).

Discussion Thioredoxin 1 is a small multifunctional protein, predominantly found in the cytosol, that contains a redox-active dithiol / disulfide and carries various biological functions related to cell proliferation, cytoprotection against oxidative and nitrative stress, and apoptosis.[9]

In this study, patients with STEMI tended to have higher levels of TRX1 compared with controls 1 hour after pPCI, although the difference did not reach significance. Out of earlier studies, only Soejima et al[8] assessed serial levels of TRX in patients with acute myocardial infarction at admission, then 12 hours and 1, 2, and 4 weeks after admission. Higher levels of TRX on admission were an independent risk factor for failure of reperfusion therapy. Shim et al[10] showed that the pre-PCI serum TRX1 levels correlated with myocardial damage in patients with STEMI, and thus were indirectly associated with patient prognosis.

8-isoprostanes are a family of compounds produced from arachidonic acid in membrane phospholipids via a free radical–catalyzed mechanism. They have been used as the most reliable markers of in vivo lipid peroxidation resulting from oxidative stress, such as ischemia and reperfusion in the heart[3,11] and other tissues.

In our study, the baseline levels of 8-ISO were higher in pPCI patients than in controls, and significant decrease was observed 6 hours following pPCI. This pattern is consistent with previous studies, for example, by Kijima et al.[11]

An association between changes in 8-ISO and patient outcomes has been observed previously. In a study by Ansley et al,[12] an inverse correlation was noted between the speed of decay of plasma 8-ISO concentrations during the early phase of reperfusion and postoperative cardiac functional recovery in patients undergoing coronary artery bypass surgery. Interestingly, postoperative cardiac index did not correlate with baseline isoprostane levels nor with their concentrations during ischemia. These results demonstrate the importance of assessing the changes in marker levels in contrast to measuring a concentration of a single marker.

In this study, longer time delay to reperfusion was associated with higher baseline levels of TRX1 and similar trend for 8-ISO was observed at 1 hour following pPCI. These results are consistent with the hypothesis that prolonged ischemia is associated with a higher level of oxidative stress and consequently a greater reperfusion injury. This phenomenon was described by Feng et al,[2] who demonstrated that the concentration of serum advanced oxidation protein products correlated with time from pain onset to hospital presentation and was associated with an increased incidence of major adverse cardiac events during 6-month follow-up.[2]

Conclusions To our knowledge, this is the first study to demonstrate an association between TRX1 levels and ischemic time—a major prognostic factor in patients with STEMI treated by pPCI. Moreover, it offers more detailed insight into the periprocedural dynamics of 8-ISO and TRX1 in relation to reperfusion of STEMI by pPCI.

Oxidative stress markers may have a possible role in clinical practice, such as to refine the prognostic evaluation of patients with acute myocardial infarction, treatment monitoring, or a supportive antioxidant treatment to overcome negative effects of reperfusion injury and longer ischemic time. Changes in marker levels in relation to ischemic time and reperfusion seem more relevant than isolated values.

SUPPLEMENTARY MATERIAL

Supplementary material is available at www.mp.pl/paim.

ARTICLE INFORMATION

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CONFLICT OF INTEREST None declared.

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