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Glutathione infusion before primary percutaneous coronary intervention: a randomized controlled trial.

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Abstarct

Objective In the setting of reperfused ST-elevation myocardial infarction (STEMI), increased production of reactive oxygen species (ROS) contributes to reperfusion injury. Among ROS, hydrogen peroxide (H_2O_2) showed toxic effects on human cardiomyocytes and may induce microcirculatory impairment. Glutathione(GSH) is a water-soluble tripeptide with a potent oxidant scavenging activity. We hypothesized that the infusion of GSH before acute reoxygenation might counteract the deleterious effects of increased H_2O_2 generation on myocardium.

Methods Fifty consecutive STEMI patients scheduled to undergo primary angioplasty were randomly assigned, before intervention, to receive an infusion of GSH (2500 mg/25ml over 10 min) followed by drug administration at the same doses at 24, 48, 72 hours elapsing time or placebo. Peripheral blood samples were obtained before and at the end of procedure as well as after 5 days. H_2O_2 production, 8-iso-PGF2α formation, H_2O_2 breakdown activity (HBA) and nitric oxide (NO) bioavailability were determined. Serum cardiac-Troponin T (cTpT) was measured at admission and up to 5 days.

Results Following acute reperfusion, a significant reduction of H_2O_2 production (p=0.0015) and 8-isoPGF2α levels (p=0.0003) as well as a significant increase in HBA (p=0.000001) and NO bioavailability (p=0.035) was found in the GSH group as compared with placebo. In treated patients, attenuated production of H_2O_2 persisted up to 5 days from the index procedure (p=0.009) and was linked to progressive decrease of cTpT levels (r=0.41, p= 0.023).

Conclusion The prophylactic and prolonged infusion of GSH determined a rapid onset and persistent blunting of H_2O_2 generation with positive benefits on myocardial cell survival.

Key words: Glutathione; STEMI; Reperfusion Injury; Reactive Oxygen Species; hydrogen peroxide.

Article summary

Strengths and limitations of this study

1. In patients who suffer from STEMI, acute reoxygenation of ischemic myocardium can induce additional myocardial cell injury mainly driven by heightened oxidative status.
2. Reactive oxygen species (ROS) generation further contributes to damage myocardium by limiting bioavailability of nitric oxide at microcirculatory level.
3. This pilot study demonstrates for the first time that in the setting of STEMI reperfusion the rapid onset and prolonged antioxidant (scavenging) activity obtained by infusion of GSH protects the myocardium.
4. This study is limitated for the lack of clinical end points, the small sample size Moreover, the absence of morphologic imaging, the qualitative evaluation of GSH-induced improvement of myocardial reperfusion indexes, as assessed in our study, might only represent the effect of a preserved microcirculatory responsiveness to vasoactive substances (i.e. NO) but unable to limit the expansion of myocardial cell damage.

Introduction

It is well known that reactive oxygen species (ROS) are produced at an accelerated rate in tissues subjected to reperfusion and that the accumulation of ROS contributes to reperfusion injury during reintroduction of molecular oxygen to the ischemic environment.[1,2]

In the setting of ST-Elevation Myocardial Infarction (STEMI), ROS-induced myocardial cell death occurs in the first few minutes of acute reoxygenation[3] and may continue for weeks to months by activation of apoptosis and autophagy processes.[4,5] ROS generation also contributes to structural capillary damage and endothelial dysfunction, which hinder the achievement of an optimal perfusion grade at microcirculatory level.[6,7] Over the time, this may result in adverse left ventricular (LV) remodeling and worse LV function.

Among ROS, hydrogen peroxide (H_2O_2) shows an important role in ischemia/reperfusion damage. In particular, the exposure of cultured human cardiomyocytes to H_2O_2 has determined rapid onset and progressive oxidative cell death.[8] Moreover, H_2O_2 influences platelet activation and promotes vascular dysfunction through thromboxane A_2 and isoprostanes formation, which are vasoconstrictors and powerful aggregating molecules derived from lipid peroxidation of esterified unsaturated fatty acids.[9-11]

Human possesses numerous enzymatic and non-enzymatic antioxidant systems. Among enzymatic system, glutathione peroxidase (GPx) plays an important role to prevent potentially deleterious effects of H_2O_2.[12] Thus, the reduced plasma level of glutathione (GSH), a water-soluble tripeptide with a potent oxidant scavenging activity and fundamental substrate for GPx activity, could have a key role in promoting myocardial and endothelial cell damage.[13] In fact, a decrease in myocardial GSH content has been observed during ischemia and reperfusion of the ischemic myocardium.[14]

Despite robust evidences regarding the role of ROS in reperfusion injury, currently, in clinical practice, there are no treatments aimed to prevent ROS generation. According to our previous hypothesis,[15] during reperfusion of ST-elevation myocardial infarction (STEMI), the use of GSH

might counteract deleterious effect of augmented oxidant activity. Therefore, we performed a pilot *proof-of-concept* study to explore whether intravenous GSH administration, just before reopening of infarct related artery and after effective reperfusion, is able to attenuate the cytotoxic activity of H_2O_2 on myocardium.

Methods

Study Design

GSH2014 (EurodraCT number 2014-004486-25) is a multicenter, no profit, randomized, double-blind, prospective, placebo-controlled trial. The Department of Heart and Great Vessel "A. Reale", Sapienza University of Rome, Italy was the coordinator centre. The study has been planned according to principles of the declaration of Helsinki. Italian Medicines Agency (AIFA) authorization and single Ethic Committee approval has been obtained for all the centers participating the study (n=3). The coordinating center designated the protocol. An external Core Lab processed the data. Written informed consent was obtained from all patients enrolled.

Study population and protocol

Between March and August 2017, we screened 157 consecutive STEMI patients, age >18 years, both sexes, referred to the hospitals of the centre of Italy belonging to our working group for primary percutaneous coronary intervention (pPCI). Inclusion criteria were: typical chest pain lasting more than 30 min with pain onset <12 h, ST segment elevation >0.2 mV in at least two contiguous leads in the initial ECG, successful pPCI (residual coronary stenosis <20%) and blood sampling for biochemical determinations collected prior to pPCI. Exclusion criteria were: symptoms duration > 12 h (n=15), rescue PCI (n=16), cardiogenic shock (n=3), left main disease (n=3), evidence of coronary collateral vessels (Rentrop score of 2 or 3 for the area at risk) (n=5), prior myocardial infarction (n=7), estimated glomerular filtration rate less than 30 ml/min (n=13), acute infection (n=2), treatment with systemic corticosteroids (n=4) or oral anticoagulants (n=7), malignancy (n=3), in-stent thrombosis (n=3), lack of consent to participate (n=18). Additionally, 8

patients were ineligible because no blood samples were collected before the start of procedure. Finally, a total of 50 patients were enrolled.

After percutaneous access was obtained, an intravenous bolus of 5.000 U of unfractionated heparin was administered, with sufficient supplements (if necessary) to maintain an activated clotting time (ACT) ≥ 250 seconds during interventions. After baseline collection of peripheral blood samples, patients were randomized to an intravenous infusion of GSH (2500 mg/25 ml over 10 min) or placebo (saline solution) before pPCI. Patients underwent pPCI according to standard protocols. The use of thrombus aspiration, glycoprotein IIb/IIIa inhibition was left to the discretion of the treating physician. Multivessel PCI was performed in a staged fashion (7 to 10 days from index procedure). All patients had drug-eluting stents implanted in treated vessels. After interventions, GSH was infused at the same doses at 24, 48, 72 hours elapsing time. Further blood samples were obtained at the end of procedure and 5 days from index procedure. Serum cardiac Troponin T (cTpT) was measured at admission, before the procedure and after reperfusion every 6 h over the next 2 days, and thereafter once a day up to 5 days. Serum cTpT levels were measured using an automated enzyme immunoassay system (Thermo Scientific, code EH TNNT1) with the upper limit of normal being 0.035 ng/ml in our laboratory. The area under the curve (AUC) (expressed in arbitrary units) troponin release was measured in each patient by computerized planimetry. After 60'-90', a post-procedural 12 leads- ECG for ST measurement were performed. Corrected TIMI frame count (cTFC) and TIMI myocardial perfusion grade (TMPG) were assessed before and after pPCI as previously described.[16] The interventional cardiologists who performed angiographic evaluation were unaware of the study assignment. Digital angiograms were analyzed off-line with the use of an automated edge detection system (Cardiovascular Medical System, MEDIS Imaging Systems, Leiden, the Netherlands).

Randomisation and blinding

An individual not involved in the study assigned codes to the study treatments, randomly allocated patients to an intravenous infusion of GSH (2500 mg/25 ml over 10 min) or placebo (saline

solution) before pPCI and kept the key in a sealed envelope. The randomisation was carried out by a computer-generated random sequence. The authors and laboratory technicians were unaware of the treatment allocation.

Primary Endpoint

GSH prophylactic infusion at the time of pPCI followed by drug administration up to 3 days after procedure would attenuate ROS induced myocardial damage as assessed by measuring biochemical markers of cell death.

Secondary Endpoints

GSH prophylactic infusion would improve myocardial reperfusion indexes as assessed by evaluation of cTFC and TMPG.

Peripheral blood samples

Blood samples were drawn from antecubital vein, before the start of procedure and after stent deployment in all patients and then collected into tubes without anticoagulant or with 3.8% sodium citrate, lithium heparin and EDTA and centrifuged at 300×g for 10 min to obtain supernatant. Plasma and serum aliquots were stored at -80°C in appropriate cuvettes until assayed. Markers of oxidative stress and antioxidant system were analyzed in serum samples.

H_2O_2 production

The H_2O_2 was evaluated by a Colorimetric Detection Kit (Arbor Assays, Ann Arbor, Michigan, US) and expressed as μM. Intra-assay and inter-assay coefficients of variation were 2.1% and 3.7%, respectively.

Determination of % HBA in peripheral serum

The evaluation of the ability to detoxify H_2O_2 was assessed by the analysis of the H_2O_2 breakdown activity (HBA) in serum with HBA assay kit (Aurogene, Rome, Italy, code HPSA-50). The % of HBA was calculated according to the following formula: % Of HBA = [(Ac-As) / Ac] X 100 where Ac is the absorbance of H_2O_2 1.4 mg/ml and As is the absorbance in the presence of the serum sample.

Serum Nitric Oxide (NO) bioavailability

A colorimetric assay kit (Cell Biolabs, San Diego, CA US) was used to determine NO bioavailability by measurement of the nitric oxide metabolites nitrite and nitrate (NOx) in the serum. Intra-assay and inter-assay coefficients of variation were 2.9% and 1.7% respectively.

Serum 8-iso-Prostaglandin F2α formation

Concentration of 8-iso-PGF2α in serum was measured by validated enzyme immunoassay (EIA) method (DRG International, Springfield, NJ, USA). Intra-assay and inter-assay coefficients of variation were 5.8% and 5.0% respectively. Values were expressed as pmol/L.

Myocardial function

After 120 minutes and 5 days from the intervention, left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV) and ejection fraction (LVEF) were calculated by the biplane Simpson's rule, as recommended by the American Society of Echocardiography. The mean values of three measurements were used for statistical evaluation.

Statistical analysis

Categorical variables were reported as counts (percentage) and continuous variables as means ± standard deviation (SD). We tested the independence of categorical variables by $\chi 2$ test and the normal distribution of parameters by Kolmogorov-Smirnov test. We used Student paired and unpaired t test, repeated measure ANOVA and Pearson product-moment correlation analysis to evaluate normally distributed continuous variables. Appropriate nonparametric tests (Mann-Whitney U test, Wilcoxon rank test and Spearman rank correlation test) were employed for all the other variables. As an overall nonparametric ANOVA, the Friedman test for the analysis of intragroup variations was used. In cases of significance, we compared pair related samples using the Wilcoxon test. The intergroup analysis was performed with the nonparametric Mann–Whitney U-test. Only two-tailed probabilities were used for testing statistical significance. Probability values <

0.05 were regarded as statistically significant. All calculations were made with the computer program STATISTICA 7 (StatSoft, Tulsa, OK, USA).

Results

Population. Twenty-five patients randomly received GSH and 25 placebo. All patients completed the phases of the study and no side effects were observed during or after GSH or placebo infusion. Clinical and angiographic characteristics of patients are shown in Table 1 and 2. The baseline characteristics were well balanced between the two groups.

Oxidative stress, antioxidant status and vascular function in peripheral samples. Biochemical data are summarized in Table 3. Baseline H_2O_2 and 8-iso-PGF2α levels were similar between treated patients and controls. After PCI, a significant reduction of H_2O_2 production and 8-iso-PGF2α levels was observed in GSH group as compared to controls (Figure 1A and B). Moreover, a significant increase in HBA and NO bioavailability was observed (Figure 1C and D). At the 5 days from index procedure, a persistent significant reduction of H_2O_2 production and a sustained increase in HBA and NO bioavailability was observed in the GSH group as compared with controls (Figure 1A-D).

Serological markers of myocardial injury. Baseline cTpT mean values were similar between GSH and placebo groups (176.0 ± 20.9 pg/ml vs. 165.4± 20.9 pg/ml, p=0.079). At 12 hours and 5 days after pPCI, GSH-treated patients showed a progressive decrease of cTpT levels (170.0 ± 44.7 pg/ml and 137.9 ± 23.7 pg/ml; -21±23.1%, p=0.009 vs. baseline). Differently, a significant increase and persistence of high values of cTpT were observed in placebo group (183.0 ± 34.8 pg/ml and 181.9 ± 18.0 pg/ml; +12.4±23.1%, p=0.029 vs. baseline) (Figure 2A). A tight correlation between percentage changes of H_2O_2 and cTpT levels from baseline to 5 days was found in treated group (Figure 2B).

Myocardial Reperfusion indexes. Post procedural cTFC values did not show a statistical significant reduction between treated and control groups (20.7±7.3 vs. 23.4±5.1, p=0.156). Interestingly, 6 patients (24%) in the placebo group and 15 (60%) patients in GSH group reached

lower-risk (<=20 frames/s) cTFC class (p=0.019). After PCI, TMPG ≥ 2 was assessed in 21 patients (84%) and 14 patients (56%) of the GSH and placebo groups, respectively (p = 0.064). Of note, 11 patients (44%) of the GSH group only had TMPG=3 (p=0.0002 vs. controls). Post-reperfusion cTFC values showed a significant correlation with changes of 8-iso-PGF2α (R=0.55, p=0.012) levels from baseline.

Myocardial function. Myocardial function was not different between groups after either baseline or at discharge. There was no significant difference between groups regarding LVEF, LVEDV or LVESV at any time point, although a trend towards reduced LVED, index of left ventricular remodelling, was assessed in treated patients (Table 4).

Discussion

This pilot study demonstrates for the first time that in the setting of STEMI reperfusion the rapid onset and prolonged antioxidant (scavenging) activity obtained by infusion of GSH protects the myocardium. Data from experimental and clinical studies suggest that following reperfusion myocardial cells death largely contributes to the final infarct size.[17,18] On the other hand, the extent of damaged myocardium is the most important predictor of adverse ventricular remodelling and it is linearly dependent upon the amount of myocardial salvage by and after reperfusion. Thus, attenuation of pro-oxidant state is an important goal in cardioprotective interventions.[19] Noteworthy, the serum of GSH treated patients showed a greater capacity to detoxify H_2O_2 evaluated by the HBA, an assay that measure the percentage of H_2O_2 neutralized into the samples.[20] We found an early and considerable increase of HBA, with positive effects on myocardial cell survival. Current evidences demonstrate that hostile oxidant environment promotes cardiomyocyte death in the first few minutes of reflow suggesting the existence of a tight window of effective cardioprotection.[21,22] Therefore, ROS-induced injury may continue for weeks to months as a result of activation of programmed cell death. Our data have shown a persistent heightened oxidative status along with decreased scavenging activity in untreated patients. This behaviour makes the duration of pharmacologic interventions a central point of cardioprotection strategies. In

the present study, GSH infusion, starting just before reperfusion with subsequent administration up to 3 days after, promoted early and sustained increase of serum HBA with attenuated production of H_2O_2 which was highly related to progressive significant reduction of serological signs of myocardial injury. In the clinical setting, the efficacy of reperfusion relies on the assessment of cardiac biomarkers release, which represents the demonstration of irreversible decay of cardiac myocytes. Our data show a progressive significant decrease of serum cTpT release during the 5 days of reperfusion in the GSH-treated patients compared with the control group resulting in a 21% reduction of myocardial damage. Despite that, in our population, the systolic function was not different between groups after reperfusion, although a trend towards reduced LVEDV was observed in treated patients. A possible explanation relies on the fact that inside the area at risk variable amount of hibernated and stunned myocardium may coexist, thus affecting the prompt recovery of contractility after reperfusion.[23] Cells have a number of mechanisms for dealing with the toxic effects of oxygen. One of the most important is connected with the widely distributed tripeptide thiol glutathione.[12,24] In particular, the glutathione redox cycle is a more efficient antioxidant protective mechanism of the heart, which acts by maintaining thiol groups of enzymes and other proteins in their reduced state thus preventing cell membrane lipid peroxidation and limiting cardiomyocytes loss.[25] Furthermore, in our study, a close relation between reduced myocardial reperfusion perfusion, increased of 8-iso-PGF2α serum levels has been observed, suggesting that oxidative unbalance may be involved in microcirculation functional damage. As previously reported, impaired tissue-level perfusion develops within minutes of established acute revascularization of ischemic areas[26] and persists for at least 1 week.[27] In this context, there is robust evidence that ROS mediated isoprostanes production contributes importantly to the post-reperfusion microvascular impairment.[28,29] Current findings implement this observation by demonstrating a sustained production of isoprostanes up to 5 days after reperfusion thereby suggesting their contributory role in the pathogenesis and persistence of microvascular dysfunction that may affect myocardial cell survival. The infusion of GSH before and 24, 48, 72 hours after

pPCI reduced isoprostanes serum levels and their reduction was linked to improvement of myocardial reperfusion indexes. Moreover, the increase in extracellular peroxide oxidants may reduce bioavailability of nitric oxide that is thought to contribute to promoting platelet hyperactivity and vasoconstriction.[9] In our study, GSH supplementation seems to have a primary role in preserving NO bioavailability and its vasodilator capacity at microcirculatory level.

This study has implications and limitations. Although the positive effects on reperfusion indexes and biochemical signs of myocardial necrosis suggest the value of prophylactic and prolonged GSH administration in preventing reperfusion injury, the lack of clinical end points and the small sample size limit the readiness of the study for clinical purposes. In addition, at discharge LVEF did not change between treated patients and controls thereby limiting the possibility to translate biochemical and angiographic benefits into improvement of prognosis.

A further concern regards the lack of quantitative assessment of both infarct size and microvascular obstruction extent. Within a defined area at risk, the manifestations of ischemia-reperfusion vascular injury go from reversible functional impairments to irreversible structural damage and contribute to final amount of infarct myocardium. In absence of morphologic imaging, the qualitative evaluation of GSH-induced improvement of myocardial reperfusion indexes, as assessed in our study, might only represent the effect of a preserved microcirculatory responsiveness to vasoactive substances (i.e. NO) but unable to limit the expansion of myocardial cell damage. Indeed, other mechanisms, such as interstitial edema and inflammatory reaction, which induce a sustained impairment of microvascular perfusion, may primarily act to increase the amount of irreversible injured myocardium thus promoting adverse ventricular remodelling.

Although in our study the reduction of infarct size and improvement of microcirculatory reperfusion indexes go in parallel, the use of high spatial resolution techniques could allow to better explore the potential of antioxidant glutathione targeting both myocardial and coronary microvascular compartment.

In conclusion, we have shown that prophylactic and prolonged GSH infusion mitigates the negative effects of the excessive and persistent H_2O_2 formation on myocardial cells. Therefore, in patients undergoing pPCI the infusion of a powerful antioxidant scavenger, such as GSH, may be useful to improve microcirculatory perfusion in order to further blunt the injury of myocardial cells. At moment, our data represent only a hypothesis generating observation that requires larger STEMI population and prolonged follow-up to confirm the role of GSH administration as cardioprotective therapy.

Contributors

GT, EM led on the conception, design and writing of the study and study protocol with substantial contributions to the design, writing, critical review of intellectual content; GT, AA, AP, NV, RM, enrollment of patients; RC, VC, CN, provided laboratory analyses; VR, SB, provided further essential statistical advice and expertise on the study protocol; FB, LL, MP, CG, providing expert clinical support; AG, MD, made substantial contributions to the trial design and management.

Ethics and dissemination

Italian Medicines Agency (AIFA) authorization and single Ethic Committee approval has been obtained for all the centers participating the study. Dissemination of results will be via peer-reviewed research publications both online and in print, conference presentations, posters, patient forums and Trust bulletins.

Data Availability

The data used to support the findings of this study are included within the article.

A data sharing statement

No additional data available

Conflicts of Interest

Authors declare that there are not conflicts of interest.

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Table 1. Clinical characteristics of the study population

Variables	GSH group (n=25)	Placebo group (n=25)	P value
Age (y, mean±SD)	66 ± 10.7	66.9 ± 9.1	0.74
Male, n (%)	15 (60)	13 (52)	0.98
Body-mass index§ (mean+SD)	26.9 ± 3.9	20 ± 3.8	0.38
Killip class ≥ 3, n (%)	2 (80)	0 (0)	0.47
Diabetes Mellitus, n (%)	5 (20)	5 (20)	1
Hypertension, n (%)	14 (56)	17 (68)	0.56
Dyslypidemia, n (%)	11 (44)	13 (52)	0.77
Statin use, n (%)	8 (32)	8 (32)	1
Smokers, n (%)	17 (68)	13 (52)	0.38

§ The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Angiographic parameters

Variables	GSH group (n=25)	Placebo group (n=25)	P value
Ischemia time (min; mean ± SD)	286 ± 88	270 ± 96	0.85
Thrombus Burden ≥ 3, n (%)	12 (48)	11 (44)	0.77
Thrombus aspiration, n (%)	13 (52)	12 (48)	0.87
GP IIb/IIIa inhibitors, n (%)	2 (8)	3 (12)	0.63
MVD, n (%)	13 (52)	11 (44)	0.77
2 vessels, **3 vessels**, **Staged PCI**, n (%)	8 (32) 5 (20) 9 (36)	5 (20) 6 (24) 5 (20)	0.89
IRA:			
LAD, n (%)	10 (40)	9 (36)	0.77
LCx, n (%)	5 (20)	6 (24)	0.73
RCA, n (%)	10 (40)	10 (40)	1
cTFC after PCI (frames/sec, mean±SD)	20.7± 7.3	23.4 ± 5.1	0.156
cTFC < 20 frames/sec, n (%)	15 (60)	6 (24)	0.019
MPG after PCI ≥2, n (%)	21 (64)	14 (48)	0.064

MPG after PCI = 3, n (%)	11 (44)	0 (0)	0.002

PCI = percutaneous coronary intervention; **IRA** = infarct related coronary artery; **LAD** = left anterior descending coronary artery**; LCx** = left circumflex coronary artery; **RCA** = Right coronary artery; **MPG** = myocardial perfusion grade; **cTFC** = corrected TIMI frame count.

Table 3. Biochemical data

	Baseline			**Reperfusion**			**Follow-up (5 days)**		
Variable	**GSH**	**Placebo**	**p**	**GSH**	**Placebo**	**p**	**GSH**	**Placebo**	**p**
H_2O_2 µM, mean±SD	40.6±8.4	43.6±11.6	0.305	28.4±12	42.8±14.1	0.0003	24±7	39.5±17.3	0.0001
8-iso-PGF2α pmol/L, mean±SD	214.6±81.1	211.9±92.1	0.91	163.6±44.7	217.6±51.6	0.0003	159.9±34.2	213.1±50.9	0.0001
HBA %, mean±SD	43.6±7.4	43.4±11.9	0.94	57.9±8.6	43.9±8.7	0.0001	62.9±10.5	45.2±13.0	0.0001
NO µM, mean±SD	16.3±5.7	16.5±4.7	0.89	27.7±7,2	22.4±10	0.0356	35.5±8.1	23.5±15.5	0.0013

GSH = reduced Glutathione; **H_2O_2** = Hydrogen Peroxide; **8-iso-PGF2α** = 8-iso-Prostaglandin F2α; **HBA** = H_2O_2 break-down activity

Table 4. Left Ventricular echocardiographic parameters at baseline and at follow-up

Echo parameters	Placebo (n= 25)	GSH (n=25)	*P*-value*
Baseline			
LVEDV (mL/m2)	121.3 ± 17.2	124.4 ± 22.3	0.44
LVESV (mL/m2)	65.4 ± 11.3	66.3± 13.2	0.91
LVEF (%)	47.5 ± 4.9	46.9 ± 4.8	0.42
Follow-up			
LVEDV (mL/m2)	118.1 ± 17.8	113.2 ± 14.1	0.42
LVESV (mL/m2)	60.9 ± 10.7	58.8± 12.5	0.91
LVEF (%)	49.1 ± 3.2.0	49.8 ± 3.7	0.42

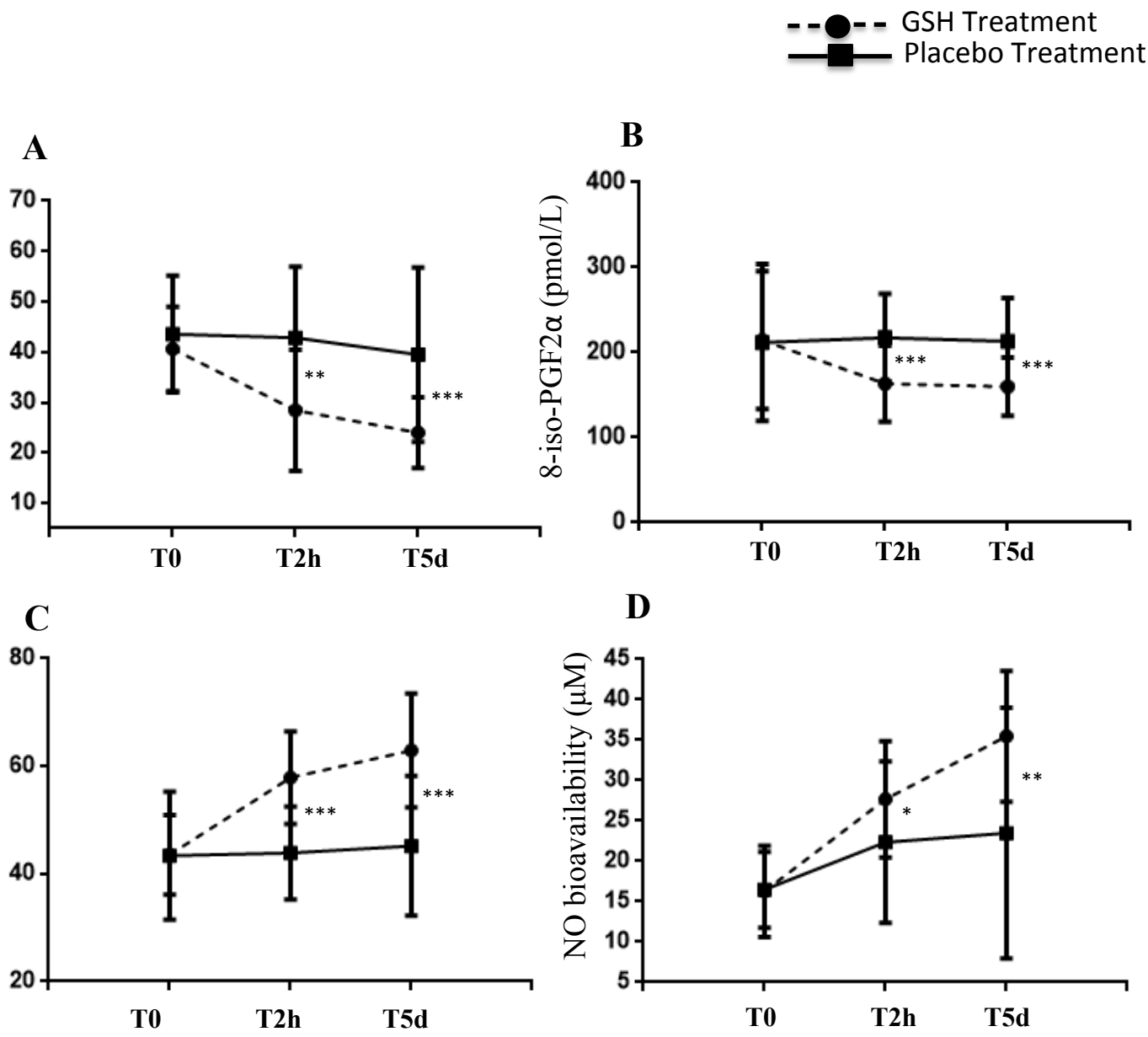
LVEDV = left ventricular end-diastolic volume; **LVEDSV** = left ventricular end-Systolic volume; **LVEF** = left ventricular ejection fraction-diastolic volume

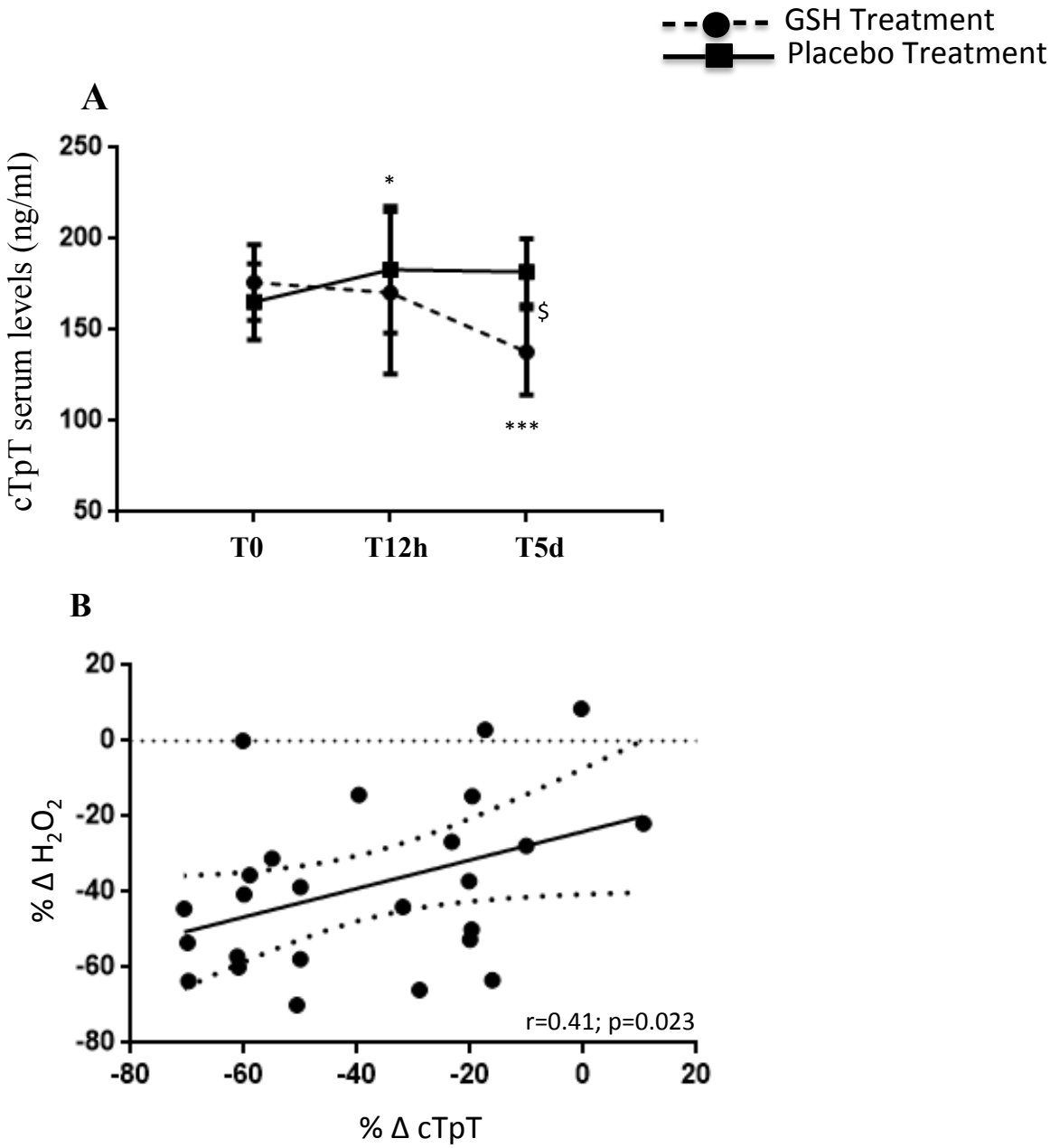
Figure Legend

Figure 1. H_2O_2 production (A), 8-iso-PGF2α formation (B), hydrogen peroxide breakdown activity (HBA) (C) and NO bioavailability (D) at baseline, after 2 hours (T2h) and at the 5 days (T5d) from the PCI in patients received GSH (n=25, dashed line) or placebo (n=25, continuous line) (***p <0.001, **p <0.01, *p <0.05).

Figure 2. cTpT levels at baseline, after 12 hours (T12h) and at the 5 days (T5d) from the PCI in patients received GSH (n=25, dashed line) or placebo (n=25, continuous line) (A) (***p<0.0001 vs. T0, *p<0.05 vs. T0, $^{\$}$p<0.05 between groups).

Linear correlation between % Δ H_2O_2 and % Δ cTpT in GSH treated group (B).


GSH Treatment
Placebo Treatment
A
B
C
D
H2O2 production (μM)
8-iso-PGF2α (pmol/L)
HBA (%)
NO bioavailability (μM)
T0
T2h
T5d
**
***
*


GSH Treatment
Placebo Treatment
A
cTpT serum levels (ng/ml)
250
200
150
100
50
*
$
***
T0
T12h
T5d
B
% Δ H_2O_2
20
0
-20
-40
-60
-80
r=0.41; p=0.023
-80
-60
-40
-20
0
20
% Δ cTpT

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	6
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	NA
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	6-7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	6-7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	6-7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	6-7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	8
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	8
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	8
	13b	For each group, losses and exclusions after randomisation, together with reasons	NA
Recruitment	14a	Dates defining the periods of recruitment and follow-up	5
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	8 and Table 1
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	8
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	8 and 9
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	8 and 9
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	NA
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	11
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	12
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	9-12
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	5
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	NA

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

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Glutathione infusion before primary percutaneous coronary intervention: a randomized controlled pilot study.

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Glutathione infusion before primary percutaneous coronary intervention: a randomized controlled pilot study.

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Abstract

Objective- In the setting of reperfused ST-elevation myocardial infarction (STEMI), increased production of reactive oxygen species (ROS) contributes to reperfusion injury. Among ROS, hydrogen peroxide (H_2O_2) showed toxic effects on human cardiomyocytes and may induce microcirculatory impairment. Glutathione (GSH) is a water-soluble tripeptide with a potent oxidant scavenging activity. We hypothesized that the infusion of GSH before acute reoxygenation might counteract the deleterious effects of increased H_2O_2 generation on myocardium.

Methods- Fifty consecutive STEMI patients scheduled to undergo primary angioplasty were randomly assigned, before intervention, to receive an infusion of GSH (2500 mg/25ml over 10 min) followed by drug administration at the same doses at 24, 48, 72 hours elapsing time or placebo. Peripheral blood samples were obtained before and at the end of procedure as well as after 5 days. H_2O_2 production, 8-iso-PGF2α formation, H_2O_2 breakdown activity (HBA) and nitric oxide (NO) bioavailability were determined. Serum cardiac-Troponin T (cTpT) was measured at admission and up to 5 days.

Results- Following acute reperfusion, a significant reduction of H_2O_2 production ($p=0.0015$) and 8-iso-PGF2α levels ($p=0.0003$) as well as a significant increase in HBA ($p<0.0001$)and NO bioavailability ($p=0.035$) was found in the GSH group as compared with placebo. In treated patients, attenuated production of H_2O_2 persisted up to 5 days from the index procedure ($p=0.009$) and these changes was linked to those of cTpT levels ($r=0.41$, $p= 0.023$).

Conclusion The prophylactic and prolonged infusion of GSH seems determined a rapid onset and persistent blunting of H_2O_2 generation improving myocardial cell survival. Nevertheless, a larger trial, adequately powered for evaluation of clinical endpoints, is ongoing to confirm the current finding.

Key words: Glutathione; STEMI; Reperfusion Injury; Reactive Oxygen Species; hydrogen peroxide, Percutaneous Coronary Interventions.

Article summary

Strengths and limitations of this study

1. In patients who suffer from STEMI, acute reoxygenation of ischemic myocardium can induce additional myocardial cell injury mainly driven by heightened oxidative status.
2. Reactive oxygen species (ROS) generation further contributes to damage myocardium by limiting bioavailability of nitric oxide at microcirculatory level.
3. This pilot study demonstrates that in the setting of STEMI reperfusion the rapid onset and prolonged antioxidant (scavenging) activity obtained by infusion of glutathione (GSH) protects the myocardium.
4. This study is limited by the lack of clinical end points and the small sample size. Moreover, qualitative assessment of GSH-induced improvement of myocardial reperfusion indexes, might only represent the effect of a preserved microcirculatory responsiveness to vasoactive substances (i.e. NO) but unable to limit the expansion of myocardial cell damage.

Introduction

It is well known that reactive oxygen species (ROS) are produced at an accelerated rate in tissues subjected to reperfusion and that the accumulation of ROS contributes to reperfusion injury during reintroduction of molecular oxygen to the ischemic environment.[1,2]

In the setting of ST-elevation myocardial infarction (STEMI), ROS-induced myocardial cell death occurs in the first few minutes of acute reoxygenation[3] and may continue for weeks to months by activation of apoptosis and autophagy processes.[4,5] ROS generation also contributes to structural capillary damage and endothelial dysfunction, which hinder the achievement of an optimal perfusion grade at microcirculatory level.[6,7] Over the time, this may result in adverse left ventricular (LV) remodeling and worse LV function.[8-10]

Among ROS, hydrogen peroxide (H_2O_2) is produced by many enzymes including for example xanthine oxidase, lipoxygenase and, in particular, NADPH oxidase.[11] H_2O_2 shows an important role in ischemia/reperfusion damage. In particular, the exposure of cultured human cardiomyocytes to H_2O_2 has determined rapid onset and progressive oxidative cell death.[12] Moreover, H_2O_2 influences platelet activation and promotes vascular dysfunction through thromboxane A_2 and isoprostanes formation, which are vasoconstrictors and powerful aggregating molecules derived from lipid peroxidation of esterified unsaturated fatty acids.[13-15]

Human possesses numerous enzymatic and non-enzymatic antioxidant systems. Among enzymatic system, glutathione peroxidase (GPx) plays an important role to prevent potentially deleterious effects of H_2O_2.[16] Thus, the reduced plasma level of glutathione (GSH), a water-soluble tripeptide with a potent oxidant scavenging activity and fundamental substrate for GPx activity, could have a key role in promoting myocardial and endothelial cell damage.[17] In fact, a decrease in myocardial GSH content has been observed during ischemia and reperfusion of the ischemic myocardium.[18]

Despite robust evidences regarding the role of ROS in reperfusion injury, currently, in clinical practice, there are no treatments aimed at preventing ROS generation.

Preclinical study of ischemia/reperfusion demonstrated that timely application of GSH provides better cardio-protection at higher doses [19]. Our hypothesis is that the use of GSH might counteract deleterious effect of augmented oxidant activity during reperfusion of STEMI [20]. Currently, a glutathione solution is available for intravenous usage to reduce side effects of chemotherapy treatment for cancer with a tolerable safety profile, however it has never been tested in the setting of patients with STEMI.

Therefore, we performed a pilot study to explore whether a short-term intravenous GSH administration, just before and after a primary percutaneous coronary intervention (p-PCI) in STEMI patients, was able to reduce oxidative stress and antioxidant status markers, resulting in a reduction of the myocardial damage.

Methods

Study Design

GSH2014 is a multicenter, no profit, randomized, double-blind, prospective, placebo-controlled trial. The Department of Heart and Great Vessel "A. Reale", Sapienza University of Rome, Italy was the coordinator center and designed the protocol (see Supplementary file). Two other centers, "Santa Maria" Terni Hospital and "San Giovanni Evangelista" Tivoli Hospital, both in Italy, were involved in the study as recruiting site.

The study has been planned according to principles of the declaration of Helsinki. Ethic Committee of the coordinator centre and Italian Medicines Agency (AIFA) (Date of Competent Authority Decision: 2015-01-13) authorized the study**.** Written informed consent was obtained from all patients enrolled. (https://www.clinicaltrialsregister.eu/ctr-search/trial/2014-004486-25/IT#N)

Patient and Public Involvement

Patients and or public were not involved in the different stages of the study (including the design and the recruitment phase). However, we intend to disseminate the main results to trial participants

and will seek patient and public involvement in the development of an appropriate method of dissemination.

Study population and protocol

Between March and August 2017, 157 consecutive STEMI patients, age >18 years, both sexes, referred to the three enrolling centers for primary percutaneous coronary intervention (p-PCI) were screened to enter in the study. Inclusion criteria were: typical chest pain lasting more than 30 min with pain onset <12 h, ST segment elevation >0.2 mV in at least two contiguous leads in the initial ECG, successful p-PCI (residual coronary stenosis <20%) and blood sampling for biochemical determinations collected prior to p-PCI.

Exclusion criteria were: symptoms duration > 12 h (n=15), rescue PCI (n=16), cardiogenic shock (n=3), left main disease (n=3), evidence of coronary collateral vessels (Rentrop score of 2 or 3 for the area at risk) (n=5), prior myocardial infarction (n=7), estimated glomerular filtration rate less than 30 ml/min (n=13), acute infection (n=2), treatment with systemic corticosteroids (n=4) or oral anticoagulants (n=7), malignancy (n=3), in-stent thrombosis (n=3), lack of consent to participate (n=18). Additionally, 8 patients were ineligible because no blood samples were collected before the start of procedure. Finally, a total of 50 patients were enrolled (see Figure 1- CONSORT diagram). The present analysis reported the results of the interim analysis (pre-planned in the protocol) on the acute effect of GSH infusion on markers of oxidative stress.

After percutaneous access was obtained, an intravenous bolus of 5.000 U of unfractionated heparin was administered, with sufficient supplements (if necessary) to maintain an activated clotting time (ACT) ≥ 250 seconds during interventions.

After baseline collection of peripheral blood samples, patients were randomized to an intravenous infusion of GSH (2500 mg/25 ml of Glutathione Sodium Salt, Biomedica Foscama Group, Rome, Italy) or placebo (saline solution) over 10 min before p-PCI. The two solutions appeared identical in size and colour to ensure blinding. Study participants, investigators and the laboratory staff

remained blinded until the statistical analysis was performed by an independent researcher who was not involved in the study.

Patients underwent p-PCI according to standard protocols. The use of thrombus aspiration, glycoprotein IIb/IIIa inhibition was left to the discretion of the treating physician. Multivessel PCI was performed in a staged fashion (7 to 10 days from index procedure).

All patients had drug-eluting stents implanted in treated vessels. After interventions, GSH was infused at the same doses at 24, 48, 72 hours elapsing time. Further blood samples were obtained at the end of procedure and 5 days from index procedure.

After 60'-90', a post-procedural 12 leads- ECG for ST measurement were performed.

Corrected TIMI frame count (cTFC) and TIMI myocardial perfusion grade (TMPG) were assessed after pPCI as previously described [21]. An external Core Lab processed the data (G.P and G.P: independent cardiologists). Digital angiograms were analyzed off-line with the use of an automated edge detection system (Cardiovascular Medical System, MEDIS Imaging Systems, Leiden, the Netherlands).

Randomization and blinding

An individual not involved in the study assigned codes (using a computer-generated random sequence) to the study treatment with a random allocation of patients to an intravenous infusion of GSH (2500 mg/25 ml over 10 min) or placebo (saline solution) before p-PCI. The interventional cardiologists who performed p-PCI, those who analyzed digital angiograms and the laboratory technicians were unaware of study treatment allocation.

Primary Endpoint

The primary endpoint was the change on oxidative stress markers levels after 2 hours from p-PCI in patients treated with GSH as compared with placebo.

Secondary Endpoints

The secondary endpoints included the assessment of: (i) changes of oxidative stress markers levels after 5 days from the p-PCI in patients received GSH or placebo; (ii) changes in serum cTpT,

biochemical markers of myocardial cell damage, in patients received GSH or placebo before and after 5 days from the procedure.

Peripheral blood samples

Blood samples were drawn from antecubital vein, before the start of procedure and after stent deployment in all patients and then collected into tubes without anticoagulant or with 3.8% sodium citrate, lithium heparin and EDTA and centrifuged at 300×g for 10 min to obtain supernatant. All plasma and serum aliquots were stored at -80°C in appropriate cuvettes until assayed.

Markers of oxidative stress and antioxidant system (i.e. H_2O_2, H_2O_2 breakdown activity (HBA) and 8-iso-PGF2α) were analyzed in serum samples collected before p-PCI, 2 hours and 5 days after p-PCI. Due to the chemical properties of the oxidative stress markers, to avoid a long-time storage of blood samples and guarantee the laboratory test quality the analyses were performed within 6 months from the collection.

Serum cardiac Troponin T (cTpT) was measured at admission, before the procedure, 6 and 12 hours after reperfusion, and thereafter once a day up to 5 days. Serum cTpT levels were measured using ELISA Kit (Elabsciences).

H_2O_2 production

The H_2O_2 was evaluated by a Colorimetric Detection Kit (Arbor Assays, Ann Arbor, Michigan, US) and expressed as μM. Intra-assay and inter-assay coefficients of variation were 2.1% and 3.7%, respectively.

Determination of % HBA in peripheral serum

The evaluation of the ability to detoxify H_2O_2 was assessed by the analysis of **the HBA** in serum with HBA assay kit (Aurogene, Rome, Italy, code HPSA-50). The % of HBA was calculated according to the following formula: % of HBA = [(Ac-As) / Ac] X 100 where Ac is the absorbance of H_2O_2 1.4 mg/ml and As is the absorbance in the presence of the serum sample.

Serum Nitric Oxide (NO) bioavailability

A colorimetric assay kit (Cell Biolabs, San Diego, CA, US) was used to determine NO bioavailability by measurement of the nitric oxide metabolites nitrite and nitrate (NOx) in the serum. Intra-assay and inter-assay coefficients of variation were 2.9% and 1.7% respectively.

Serum 8-iso-Prostaglandin F2α formation

Concentration of 8-iso-PGF2α in serum was measured by validated enzyme immunoassay (EIA) method (DRG International, Springfield, NJ, USA). Intra-assay and inter-assay coefficients of variation were 5.8% and 5.0% respectively. Values were expressed as pmol/L.

Myocardial function

After 120 minutes and 5 days from the intervention, left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV) and ejection fraction (LVEF) were calculated by the biplane Simpson's rule, as recommended by the American Society of Echocardiography. The mean values of three measurements were used for statistical evaluation.

Sample Size Calculation

For the present preliminary analysis, the sample size calculation was estimated considering previous data available for 8-iso-PGF2α levels [22]. We hypothesized a mean difference of 20% in plasmatic 8-iso-PGF2-α levels measured at the end of successfully reperfusion when comparing the GSH group with the controls. We also assumed a SD of 50 pg/ml in each group. Based on these assumptions, this study needs 25 patients for each treatment arm for a power of ≥80% with a 2-sample t test at level 5%.

Statistical analysis

Categorical variables were reported as counts (percentage) and continuous variables as means ± standard deviation (SD). We tested the independence of categorical variables by $\chi 2$ test and the normal distribution of continuous variables by Kolmogorov-Smirnov test. We used Student paired and unpaired t test, repeated measure ANOVA and Pearson product-moment correlation analysis to evaluate normally distributed continuous variables. Appropriate nonparametric tests (Mann-Whitney U test, Wilcoxon rank test and Spearman rank correlation test) were employed for all the

other variables. As an overall nonparametric ANOVA, the Friedman test for the analysis of intragroup variations was used. In cases of significance, we compared pair related samples using the Wilcoxon test. The intergroup analysis was performed with the nonparametric Mann–Whitney U-test. Only two-tailed probabilities were used for testing statistical significance. Probability values < 0.05 were regarded as statistically significant. All calculations were made with the computer program STATISTICA 7 (StatSoft, Tulsa, OK, USA).

Results

Twenty-five patients randomly received GSH and 25 placebo. All patients completed the phases of the study (Figure 1). All patients had a TIMI flow grade equal to 0 or 1 requiring percutaneous treatment. Clinical and angiographic characteristics of patients are shown in Table 1 and 2. The baseline characteristics were well balanced between the two groups. In both groups, neither side effects during the infusion, nor adverse events during the short observation period were recorded.

Oxidative stress, antioxidant status and vascular function in peripheral samples. Biochemical data are summarized in Table 3. Baseline H_2O_2 and 8-iso-PGF2α levels were similar between treated patients and controls. After PCI, a significant reduction of H_2O_2 production and 8-iso-PGF2α levels was observed in GSH group as compared to controls **(Figure 2A and 2B).** Moreover, a significant increase in HBA and NO bioavailability was observed **(Figure 2C and 2D).**

At the 5 days from index procedure, a persistent significant reduction of H_2O_2 production and a sustained increase in HBA and NO bioavailability was observed in the GSH group as compared with controls **(Figure 2A-D).**

Serological markers of myocardial injury. Baseline cTpT mean values were similar between GSH and placebo groups (176.0 ± 20.9 pg/ml vs. 165.4± 20.9 pg/ml, p=0.079). At 6 hours, no changes in cTpT values were found in GSH-treated patients (172.1±27.7 pg/ml vs. baseline, p=0.065). At 12 hours and 5 days after pPCI, GSH-treated patients showed a progressive decrease of cTpT levels (170.0 ± 44.7 pg/ml and 137.9 ± 23.7 pg/ml; -21±23.1%, p=0.009 vs. baseline).

Differently, a significant increase and persistence of high values of cTpT were observed in placebo group (T6, 169.9±16.3 pg/ml, T12, 183.0 ± 34.8 pg/ml and T5d, 181.9 ± 18.0 pg/ml; +12.4±23.1%, p=0.029 vs. baseline) **(Figure 3A).** A modest correlation between percentage changes of H_2O_2 and cTpT levels from baseline to 5 days was found in treated group **(Figure 3B).**

Myocardial Reperfusion indexes. Post-procedural cTFC values did not show a statistically significant reduction between treated and control groups (20.7±7.3 vs. 23.4±5.1, p=0.156). Interestingly, 6 patients (24%) in the placebo group and 15 (60%) patients in GSH group reached lower-risk (<=20 frames/s) cTFC class (p=0.019). After PCI, TMPG ≥ 2 was assessed in 21 patients (84%) and 14 patients (56%) of the GSH and placebo groups, respectively (p = 0.064). Of note, 11 patients (44%) of the GSH group only had TMPG=3 (p=0.0002 vs. controls). Post-reperfusion cTFC values showed a significant correlation with changes of 8-iso-PGF2α (R=0.55, p=0.012) levels from baseline.

Myocardial function. Myocardial function was not different between groups after either baseline or at discharge. There was no significant difference between groups regarding LVEF, LVEDV or LVESV at any time point (Table 4).

Discussion

This pilot study demonstrates that in the setting of STEMI reperfusion the rapid onset and prolonged antioxidant (scavenging) activity obtained by infusion of GSH before and after primary PCI reduces the oxidative stress markers. The improvement of the antioxidant status resulted in a significant decrease of cardiac troponin, marker of myocardial damage.

Data from experimental and clinical studies suggest that following reperfusion myocardial cells death largely contributes to the final infarct size.[23,24] On the other hand, the extent of damaged myocardium is the most important predictor of adverse ventricular remodeling and it is linearly dependent upon the amount of myocardial salvage by and after reperfusion. Thus, attenuation of pro-oxidant state is an important goal in cardioprotective interventions.[25]

Noteworthy, the serum of GSH treated patients showed a greater capacity to detoxify H_2O_2 evaluated by the HBA, an assay that measure the percentage of H_2O_2 neutralized into the samples.[26] We found an early and considerable increase of HBA, with positive effects on myocardial cell survival**,** assessed by cTpT.

Current evidences demonstrate that oxidant environment promotes cardiomyocyte death in the first few minutes of reflow suggesting the existence of a tight window of effective cardio-protection.[27,28] Therefore, ROS-induced injury may continue for weeks to months as a result of activation of programmed cell death. Our data have shown a persistent heightened oxidative status along with decreased scavenging activity in untreated patients. This behavior makes the duration of pharmacologic interventions a central point of cardio-protection strategies. In the present study, GSH infusion, starting just before reperfusion with subsequent administration up to 3 days after, promoted early and sustained increase of serum HBA with attenuated production of H_2O_2 which was highly related to progressive significant reduction of serological signs of myocardial injury. In addition, our data show a progressive significant decrease of serum cTpT release during the 5 days of reperfusion in the GSH-treated patients compared with the control group resulting in a 21% reduction of myocardial damage. Despite that, in our population, the systolic function was not different between groups after reperfusion, although a trend towards reduced LVEDV was observed in treated patients. A possible explanation relies on the fact that inside the area at risk variable amount of hibernated and stunned myocardium may coexist, thus affecting the prompt recovery of contractility after reperfusion.[29]

Cells have a number of mechanisms for dealing with the toxic effects of oxygen. One of the most important is connected with the widely distributed tripeptide thiol glutathione.[16,30] In particular, the glutathione redox cycle is a more efficient antioxidant protective mechanism of the heart, which acts by maintaining thiol groups of enzymes and other proteins in their reduced state thus preventing cell membrane lipid peroxidation and limiting cardiomyocytes loss.[31] Furthermore, in our study, a close relation between reduced myocardial reperfusion and increased

of 8-iso-PGF2α serum levels has been observed, suggesting that oxidative unbalance may be involved in microcirculation functional damage. As previously reported, impaired tissue-level perfusion develops within minutes of established acute revascularization of ischemic areas[32] and persists for at least 1 week.[33] In this context, there is robust evidence that ROS mediated isoprostanes production contributes importantly to the post-reperfusion microvascular impairment.[22,34] Current findings implement this observation by demonstrating a sustained production of isoprostanes up to 5 days after reperfusion thereby suggesting their contributory role in the pathogenesis and persistence of microvascular dysfunction that may affect myocardial cell survival. The infusion of GSH before and 24, 48, 72 hours after pPCI reduced isoprostanes serum levels and their reduction was linked to improvement of myocardial reperfusion indexes. Moreover, the increase in extracellular peroxide oxidants may reduce bioavailability of nitric oxide that is thought to contribute to promoting platelet hyperactivity and vasoconstriction.[13] In our study, GSH supplementation seems to have a role in preserving NO bioavailability and its vasodilator capacity at microcirculatory level.

The Strengths and Limitations of the Study

The positive effects on reperfusion indexes and on biochemical signs of myocardial necrosis suggest the value of prophylactic and prolonged GSH administration in preventing reperfusion injury. Thus, in patients undergoing pPCI the infusion of a powerful antioxidant scavenger, such as GSH, may be useful to improve microcirculatory perfusion in order to further blunt the injury of myocardial cells.

Some limitations deserve to be discussed.

The small sample size of the study and the lack of morfologic assessment of both infarct size and microvascular obstruction extent between the two groups, actually, limit the clinical application of these findings. Within a defined area at risk, the manifestations of ischemia-reperfusion vascular injury go from reversible functional impairments to irreversible structural damage and contribute to final amount of infarct myocardium. In absence of morphologic imaging, the qualitative evaluation

of GSH-induced improvement of myocardial reperfusion indexes, as assessed in our study, might only represent the effect of a preserved microcirculatory responsiveness to vasoactive substances (i.e. NO) but unable to limit the expansion of myocardial cell damage. Indeed, other mechanisms, such as interstitial edema and inflammatory reaction, which induce a sustained impairment of microvascular perfusion, may primarily act to increase the amount of irreversible injured myocardium thus promoting adverse ventricular remodeling.

In conclusion, in this pilot study, we have shown that a short-term prophylactic GSH infusion mitigates the negative effects of the excessive and persistent H_2O_2 formation on myocardial cells. The findings of the present study require to be confirmed through an adequately powered STEMI population. A larger trial with a prolonged follow-up for evaluation of clinical endpoints is needed to confirm the role of GSH administration as cardioprotective therapy.

Figure Legend

Figure 1. CONSORT flowchart

Figure 2. H_2O_2 production (A), 8-iso-PGF2α formation (B), hydrogen peroxide breakdown activity (HBA) (C) and NO bioavailability (D) at baseline, after 2 hours (T2h) and at the 5 days (T5d) from the PCI in patients received GSH (n=25, dashed line) or placebo (n=25, continuous line). Data are expressed as mean±SEM (***p <0.001, **p <0.01, *p <0.05).

Figure 3. cTpT levels (A) at baseline, after 6 hours (T6h), 12 hours (T12h) and at the 5 days (T5d) from the PCI in patients received GSH (n=25, dashed line) or placebo (n=25, continuous line). Data are expressed as mean±SEM (***p<0.0001 vs. T0, *p<0.05 vs. T0, $^{\$}$p<0.05 between groups). Linear correlation between % Δ cTpT and % Δ H_2O_2 in GSH treated group (B).

Contributors

GTa, EM led on the conception, design and writing of the study with substantial contributions to the design, writing, critical review of intellectual content; GTr, AA, AP, NV, RM, enrollment of patients; RC, VC, CN, provided laboratory analyses; VR, SB, provided further essential statistical advice and expertise on the study protocol; FB, LL, MP, CG, providing expert clinical support; AG, MD, made substantial contributions to the trial design and management.

Ethics and dissemination

Italian Medicines Agency (AIFA) authorization and single Ethic Committee approval has been obtained for all the centers participating the study. Dissemination of results will be via peer-reviewed research publications both online and in print, conference presentations, posters, patient forums and Trust bulletins.

Data Availability Statement

The data set is available on request from the corresponding author.

Conflicts of Interest

Authors declare that there are not conflicts of interest.

Funding Source: No funding declared

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Table 1. Clinical characteristics of the study population

Variables	GSH group (n=25)	Placebo group (n=25)	P value
Age (y, mean±SD)	66 ± 10.7	66.9 ± 9.1	0.74
Male, n (%)	15 (60)	13 (52)	0.98
Body-mass index§ (mean+SD)	26.9 ± 3.9	20 ± 3.8	0.38
Killip class ≥ 3, n (%)	2 (8)	0 (0)	0.47
Diabetes Mellitus, n (%)	5 (20)	5 (20)	1
Hypertension, n (%)	14 (56)	17 (68)	0.56
Dyslypidemia, n (%)	11 (44)	13 (52)	0.77
Statin use, n (%)	8 (32)	8 (32)	1
Smokers, n (%)	17 (68)	13 (52)	0.38

§ The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Angiographic parameters

Variables	GSH group (n=25)	Placebo group (n=25)	P value
Ischemia time# (min; mean ± SD)	286 ± 88	270 ± 96	0.85
Thrombus Burden ≥ 3, n (%)	12 (48)	11 (44)	0.77
Thrombus aspiration, n (%)	13 (52)	12 (48)	0.87
GP IIb/IIIa inhibitors, n (%)	2 (8)	3 (12)	0.63
MVD, n (%)	13 (52)	11 (44)	0.77
2 vessels, **3 vessels**, **Staged PCI**, n (%)	8 (32) 5 (20) 9 (36)	5 (20) 6 (24) 5 (20)	0.89
IRA:			
LAD, n (%)	10 (40)	9 (36)	0.77
LCx, n (%)	5 (20)	6 (24)	0.73
RCA, n (%)	10 (40)	10 (40)	1

PCI = percutaneous coronary intervention; **IRA** = infarct related coronary artery; **LAD** = left anterior descending coronary artery**; LCx** = left circumflex coronary artery; **RCA** = Right coronary artery. #ischemia time was defined as the timing between symptom onset and balloon inflation.

Table 3. Biochemical data

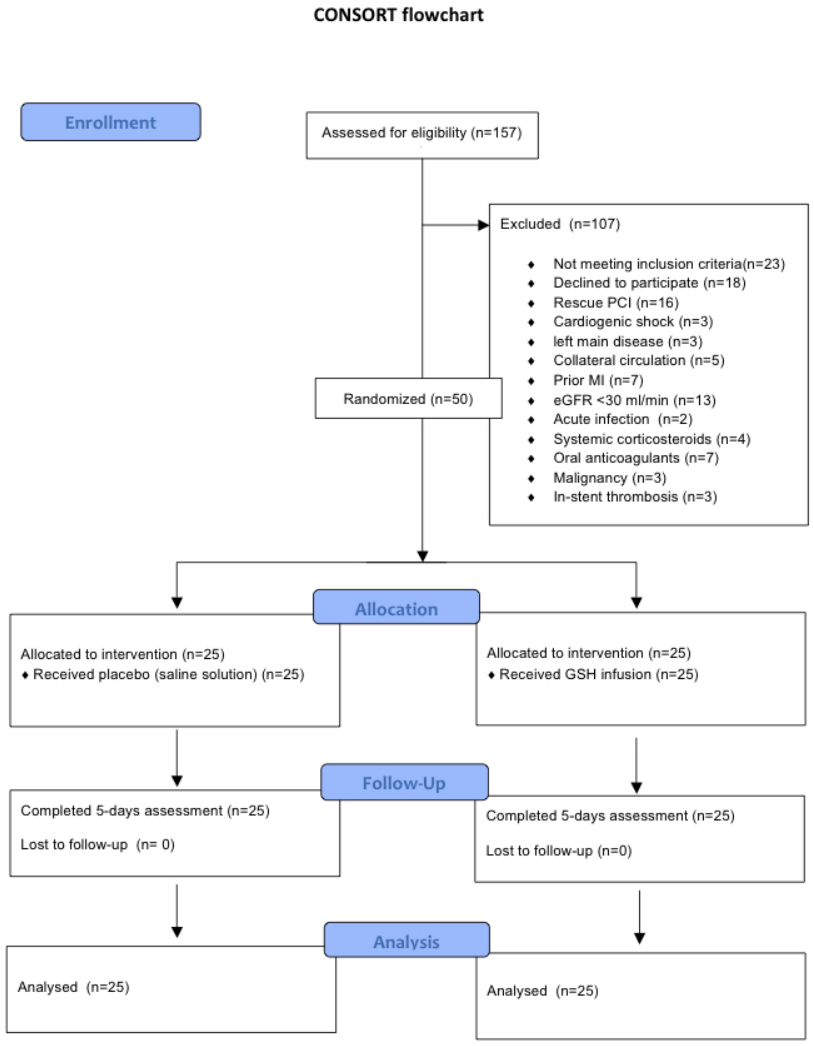
	Baseline			Reperfusion 2h			Follow-up (5 days)		
Variable	**GSH**	**Placebo**	**p**	**GSH**	**Placebo**	**p**	**GSH**	**Placebo**	**p**
H_2O_2 µM, mean±SD	40.6±8.4	43.6±11.6	0.305	28.4±12	42.8±14.1	0.0003	24±7	39.5±17.3	0.0001
Δ				-12.1±15.2	-0.7±17.9	0.03	-16.6±11.0	-4.1±20.14	0.009
8-iso-PGF2α pmol/L, mean±SD	214.6±81.1	211.9±92.1	0.91	163.6±44.7	217.6±51.6	0.0003	159.9±34.2	213.1±50.9	0.0001
Δ				-50.9±92.9	-3.3±1.29	0.02	-54.6±62.1	-1.2±115.7	0.02
HBA %, mean±SD	43.6±7.4	43.4±11.9	0.94	57.9±8.6	43.9±8.7	0.0001	62.9±10.5	45.2±13.0	0.0001
Δ				+14.9±5.5	+0.4±14.9	0.0004	+19.4±10.2	+1.8±17.1	0.0001
NO µM, mean±SD	16.3±5.7	16.5±4.7	0.89	27.7±7,2	22.4±10	0.0356	35.5±8.1	23.5±15.5	0.0013
Δ				+11.4±6.8	+5.8±10.5	0.05	+19.2±9.7	+7.0±14.7	0.002

GSH = reduced Glutathione; **H_2O_2** = Hydrogen Peroxide; **8-iso-PGF2α** = 8-iso-Prostaglandin-F2α; **HBA** = H_2O_2 break-down activity

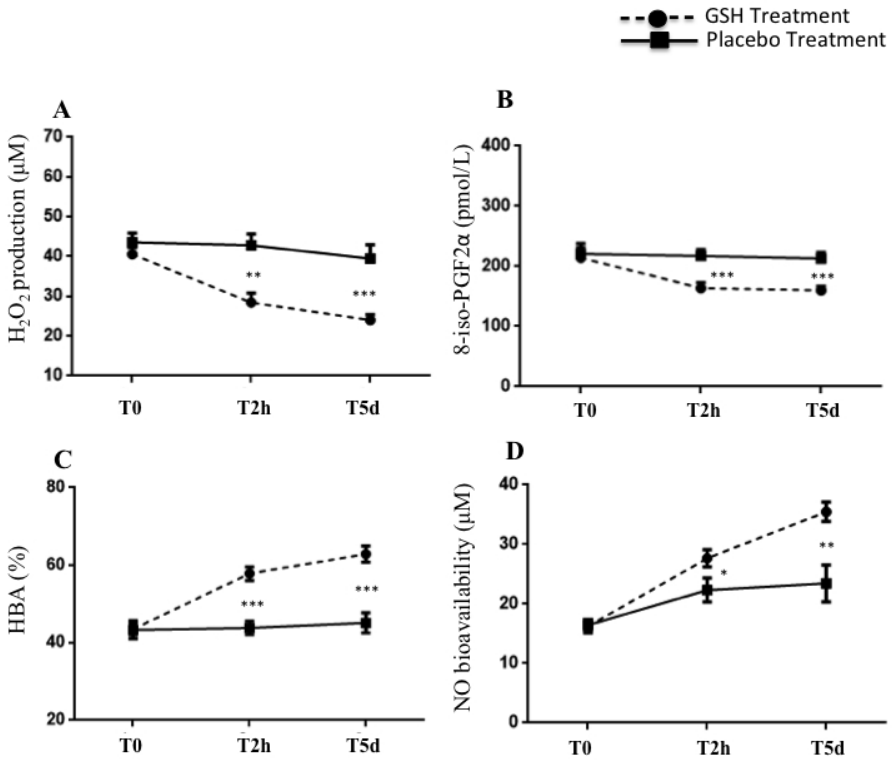
Table 4. Left Ventricular echocardiographic parameters at baseline and at follow-up

Echo parameters	Placebo (n= 25)	GSH (n=25)	*P*-value
Baseline			
LVEDV (mL/m2)	121.3 ± 17.2	124.4 ± 22.3	0.44
LVESV (mL/m2)	65.4 ± 11.3	66.3± 13.2	0.91
LVEF (%)	47.5 ± 4.9	46.9 ± 4.8	0.42
Follow-up			
LVEDV (mL/m2)	118.1 ± 17.8	113.2 ± 14.1	0.42
LVESV (mL/m2)	60.9 ± 10.7	58.8± 12.5	0.91
LVEF (%)	49.1 ± 3.2	49.8 ± 3.7	0.42

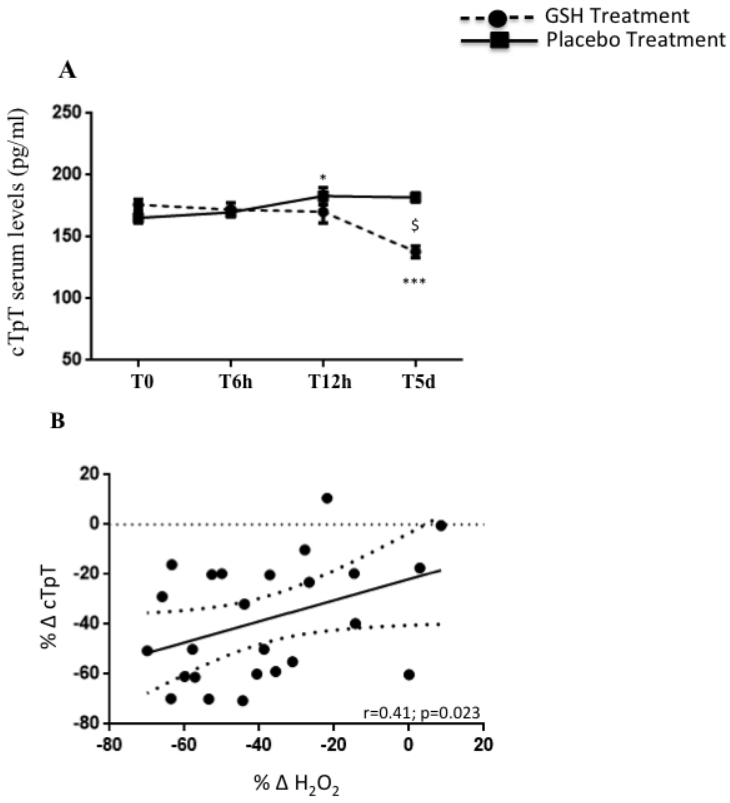
LVEDV = left ventricular end-diastolic volume; **LVEDSV** = left ventricular end-systolic volume; **LVEF** = left ventricular ejection fraction-diastolic volume



254x338mm (72 x 72 DPI)



254x338mm (72 x 72 DPI)



254x338mm (72 x 72 DPI)

EudraCT Number: 2014-004486-25
Sponsor's Protocol Code Number: GSH2014
National Competent Authority: Italy - Italian Medicines Agency
Clinical Trial Type: EEA CTA
Trial Status: submitted data of the pilot study. The trial is ongoing.
Date on which this record was first entered in the EudraCT database: 2014-12-04
Link: https://www.clinicaltrialsregister.eu/ctr-search/trial/2014-004486-25/IT/

A. Protocol Information
Member State Concerned: Italy - Italian Medicines Agency
EudraCT number: 2014-004486-25
Full title of the trial: Prevention of the reperfusion myocardical damage in patients with acute myocardial infarct (STEMI) submitted to primary PCI through infusion of intravenous glutathione.
Sponsor's protocol code number: GSH2014

B. Sponsor Information
Sponsor 1: University Hospital "Policlinico Umberto I"
Name of organization providing support: University Hospital "Policlinico Umberto I", Rome, Italy.
Functional name of contact point: Enrico Mangieri, University Hospital "Policlinico Umberto I".
Viale del Policlinico, 155 – Rome, Post code: 00161, Italy
E-mail: enrico.mangieri@uniroma1.it

D. IMP Identification
IMP to be used in the trial has a marketing authorisation: Yes
Trade name: TAD
Name of the Marketing Authorisation holder: Biomedica Foscama Group S.p.A.
Country which granted the Marketing Authorisation: Italy
Pharmaceutical form: Powder and solvent for solution for infusion
Routes of administration for this IMP: Intravenous use
Information on Placebo
Pharmaceutical form of the placebo: saline solution
Route of administration of the placebo: Intravenous use

E. General Information on the Trial
Medical condition or disease under investigation
Medical condition(s) being investigated: ST-Segment Elevation Myocardial Infarction (STEMI).
Medical condition in easily understood language: acute myocardial infarct
Therapeutic area: Diseases [C] - Cardiovascular Diseases [C14]
Objective of the trial
Main objective of the trial: To verify if the intravenous infusion of "Glutathione Sodium Salt" it is able to reduce the level of oxidative state in the area of myocardial infarction.
Secondary objectives of the trial: To verify if the intravenous infusion of "Glutathione Sodium Salt" during the procedures of primary PCI it is able to limit the extension of the ischemic area, to reduce the incidence of the no-reflow, to improve the degree of myocardial blush and to decrease the indexes of suffering post-procedural ischemia (ST elevation; release of myocardial necrosis markers).
Principal inclusion criteria: STEMI patients submitted to p-PCI up to 12 hours.
Age≥18 years. Women and Men. Signed informed consent

Principal exclusion criteria:
Patients with cardiac arrest, ventricular fibrillation, cardiogenic shock, stent thrombosis, previous acute myocardial infarction, or angina within 48 hours before infarction were not included in the study. Patients with evidence of coronary collaterals (2-3 Rentrop) to the region at risk on initial coronary angiography (at the time of admission) will be excluded. Moreover, patients with EF ≤30%, impaired renal function (creatinine > 3.0 mg/dl), recipient of heart transplant, a life expectancy less than 12 months, has known allergies to aspirin, clopidogrel bisulfate, heparin, contrast media or stainless steel that cannot be managed medically were excluded.
Patient needs therapy with warfarin or currently participating in an investigational drug or another device study were not considered enrolling.

End points
Primary end point(s): The primary endopoint will consist in the assessment of the effects of the infusion of "Glutathione Sodium Salt" on the reduction of the oxidative markers and inflammation after PCI.
Timepoint(s) of clinical evaluation of this end point: before, 2 hour and 5 days from the p-PCI

Secondary end point(s): The secondary endpoint will include: (1) the assessment of the variations of the corrected TIMI frame count (cTFC) and the TIMI Myocardial Perfusion Grade (TMPG) after p-PCI; (2) the assessment of the middle values of peak of the cardiac Troponin, after the procedure; (3) to verify, through telephone contact or a programmed visit, the principal adverse clinical events as death, acute myocardial infarct, stent's thrombosis of the treated vessels or the occurrence of a new revascularization, up to 6 months after the procedure.
Medical Doctors don't have the knowledge both about the possible infusion of the Glutathione Sodium Salt, in the examined patient, then others clinical data.

Moreover, serological levels of Troponin and creatinine will be measured before the p-PCI and after the procedure (2, 6, 12 and 24 hours).
Besides, through 2D Echocardiography with Simpson's biplane method the FE will be calculate at admission and after hospital discharge.
If clinical-instrumental signs of ischemia will rise up, the patient will be submitted to a new angiography.

Definition of the end of the trial and justification where it is not the last visit of the last subject undergoing the trial: LVLS or telephonic contact
Population of Trial Subjects
Trial has subjects under 18: No
Adults (18-64 years): Yes
Number of subjects for this age range: 30
Elderly (>=65 years): Yes
Number of subjects for this age range: 60
Female: Yes
Male: Yes
Patients: Yes
Specific vulnerable populations: Yes
Women of childbearing potential not using contraception: Yes
Women of child-bearing potential using contraception: Yes

Pregnant women: No
Nursing women: No
Emergency situation: No
Subjects incapable of giving consent personally: No
Planned number of subjects to be included: 90

F. Investigator Networks to be involved in the Trial

N. Review by the Competent Authority or Ethics Committee in the country concerned
N. Competent Authority Decision: Authorised
N. Date of Competent Authority Decision: 2015-01-13
N. Ethics Committee Opinion of the trial application: Favourable
N. Date of Ethics Committee Opinion: 2015-02-12
N. Centers involved in the study: Department of Heart and Great Vessel "A. Reale", Sapienza University of Rome (coordinator centre) - "Santa Maria" Terni Hospital - "San Giovanni Evangelista" Tivoli Hospital, all in Italy.
P. End of Trial Status: analyzed as pilot study the first 50 enrolled patients. Ongoing.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9-10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	NA
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	7
	13b	For each group, losses and exclusions after randomisation, together with reasons	7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	21-22
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-11
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	10
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13-14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	Supplementary file
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.

BMJ Open

Glutathione infusion before primary percutaneous coronary intervention: a randomized controlled pilot study.

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2018-025884.R2
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Glutathione infusion before primary percutaneous coronary intervention: a randomized controlled pilot study.

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Abstract

Objective- In the setting of reperfused ST-elevation myocardial infarction (STEMI), increased production of reactive oxygen species (ROS) contributes to reperfusion injury. Among ROS, hydrogen peroxide (H_2O_2) showed toxic effects on human cardiomyocytes and may induce microcirculatory impairment. Glutathione (GSH) is a water-soluble tripeptide with a potent oxidant scavenging activity. We hypothesized that the infusion of GSH before acute reoxygenation might counteract the deleterious effects of increased H_2O_2 generation on myocardium.

Methods- Fifty consecutive STEMI patients scheduled to undergo primary angioplasty were randomly assigned, before intervention, to receive an infusion of GSH (2500 mg/25ml over 10 min) followed by drug administration at the same doses at 24, 48, 72 hours elapsing time or placebo. Peripheral blood samples were obtained before and at the end of procedure as well as after 5 days. H_2O_2 production, 8-iso-PGF2α formation, H_2O_2 breakdown activity (HBA) and nitric oxide (NO) bioavailability were determined. Serum cardiac-Troponin T (cTpT) was measured at admission and up to 5 days.

Results- Following acute reperfusion, a significant reduction of H_2O_2 production ($p=0.0015$) and 8-iso-PGF2α levels ($p=0.0003$) as well as a significant increase in HBA ($p<0.0001$)and NO bioavailability ($p=0.035$) was found in the GSH group as compared with placebo. In treated patients, attenuated production of H_2O_2 persisted up to 5 days from the index procedure ($p=0.009$) and these changes was linked to those of cTpT levels ($r=0.41$, $p= 0.023$).

Conclusion The prophylactic and prolonged infusion of GSH seems determined a rapid onset and persistent blunting of H_2O_2 generation improving myocardial cell survival. Nevertheless, a larger trial, adequately powered for evaluation of clinical endpoints, is ongoing to confirm the current finding.

Key words: Glutathione; STEMI; Reperfusion Injury; Reactive Oxygen Species; hydrogen peroxide, Percutaneous Coronary Interventions.

Article summary

Strengths and limitations of this study

1. In patients who suffer from STEMI, acute reoxygenation of ischemic myocardium can induce additional myocardial cell injury mainly driven by heightened oxidative status.
2. Reactive oxygen species (ROS) generation further contributes to damage myocardium by limiting bioavailability of nitric oxide at microcirculatory level.
3. This pilot study demonstrates that in the setting of STEMI reperfusion the rapid onset and prolonged antioxidant (scavenging) activity obtained by infusion of glutathione (GSH) protects the myocardium.
4. This study is limited by the lack of clinical end points and the small sample size. Moreover, qualitative assessment of GSH-induced improvement of myocardial reperfusion indexes, might only represent the effect of a preserved microcirculatory responsiveness to vasoactive substances (i.e. NO) but unable to limit the expansion of myocardial cell damage.

Introduction

It is well known that reactive oxygen species (ROS) are produced at an accelerated rate in tissues subjected to reperfusion and that the accumulation of ROS contributes to reperfusion injury during reintroduction of molecular oxygen to the ischemic environment.[1,2]

In the setting of ST-elevation myocardial infarction (STEMI), ROS-induced myocardial cell death occurs in the first few minutes of acute reoxygenation[3] and may continue for weeks to months by activation of apoptosis and autophagy processes.[4,5] ROS generation also contributes to structural capillary damage and endothelial dysfunction, which hinder the achievement of an optimal perfusion grade at microcirculatory level.[6,7] Over the time, this may result in adverse left ventricular (LV) remodeling and worse LV function.[8-10]

Among ROS, hydrogen peroxide (H_2O_2) is produced by many enzymes including for example xanthine oxidase, lipoxygenase and, in particular, NADPH oxidase.[11] H_2O_2 shows an important role in ischemia/reperfusion damage. In particular, the exposure of cultured human cardiomyocytes to H_2O_2 has determined rapid onset and progressive oxidative cell death.[12] Moreover, H_2O_2 influences platelet activation and promotes vascular dysfunction through thromboxane A_2 and isoprostanes formation, which are vasoconstrictors and powerful aggregating molecules derived from lipid peroxidation of esterified unsaturated fatty acids.[13-15]

Human possesses numerous enzymatic and non-enzymatic antioxidant systems. Among enzymatic system, glutathione peroxidase (GPx) plays an important role to prevent potentially deleterious effects of H_2O_2.[16] Thus, the reduced plasma level of glutathione (GSH), a water-soluble tripeptide with a potent oxidant scavenging activity and fundamental substrate for GPx activity, could have a key role in promoting myocardial and endothelial cell damage.[17] In fact, a decrease in myocardial GSH content has been observed during ischemia and reperfusion of the ischemic myocardium.[18]

Despite robust evidences regarding the role of ROS in reperfusion injury, currently, in clinical practice, there are no treatments aimed at preventing ROS generation.

Preclinical study of ischemia/reperfusion demonstrated that timely application of GSH provides better cardio-protection at higher doses [19]. Our hypothesis is that the use of GSH might counteract deleterious effect of augmented oxidant activity during reperfusion of STEMI [20]. Currently, a glutathione solution is available for intravenous usage to reduce side effects of chemotherapy treatment for cancer with a tolerable safety profile, however it has never been tested in the setting of patients with STEMI.

Therefore, we performed a pilot study to explore whether a short-term intravenous GSH administration, just before and after a primary percutaneous coronary intervention (p-PCI) in STEMI patients, was able to reduce oxidative stress and antioxidant status markers, resulting in a reduction of the myocardial damage.

Methods

Study Design

GSH2014 is a multicenter, no profit, randomized, double-blind, prospective, placebo-controlled trial. The Department of Heart and Great Vessel "A. Reale", Sapienza University of Rome, Italy was the coordinator center and designed the protocol (see Supplementary file). Two other centers, "Santa Maria" Terni Hospital and "San Giovanni Evangelista" Tivoli Hospital, both in Italy, were involved in the study as recruiting site.

The study has been planned according to principles of the declaration of Helsinki. Ethic Committee of the coordinator centre and Italian Medicines Agency (AIFA) (Date of Competent Authority Decision: 2015-01-13) authorized the study**.** Written informed consent was obtained from all patients enrolled. (https://www.clinicaltrialsregister.eu/ctr-search/trial/2014-004486-25/IT#N)

Patient and Public Involvement

Patients and or public were not involved in the different stages of the study (including the design and the recruitment phase). However, we intend to disseminate the main results to trial participants and will seek patient and public involvement in the development of an appropriate method of dissemination.

Study population and protocol

Between March and August 2017, 157 consecutive STEMI patients, age >18 years, both sexes, referred to the three enrolling centers for primary percutaneous coronary intervention (p-PCI) were screened to enter in the study. Inclusion criteria were: typical chest pain lasting more than 30 min with pain onset <12 h, ST segment elevation >0.2 mV in at least two contiguous leads in the initial ECG, successful p-PCI (residual coronary stenosis <20%) and blood sampling for biochemical determinations collected prior to p-PCI.

Exclusion criteria were: symptoms duration > 12 h (n=15), rescue PCI (n=16), cardiogenic shock (n=3), left main disease (n=3), evidence of coronary collateral vessels (Rentrop score of 2 or 3 for the area at risk) (n=5), prior myocardial infarction (n=7), estimated glomerular filtration rate less than 30 ml/min (n=13), acute infection (n=2), treatment with systemic corticosteroids (n=4) or oral anticoagulants (n=7), malignancy (n=3), in-stent thrombosis (n=3), lack of consent to participate (n=18). Additionally, 8 patients were ineligible because no blood samples were collected before the start of procedure. Finally, a total of 50 patients were enrolled (see Figure 1- CONSORT diagram). The present analysis reported the results of the interim analysis (pre-planned in the protocol) on the acute effect of GSH infusion on markers of oxidative stress.

After percutaneous access was obtained, an intravenous bolus of 5.000 U of unfractionated heparin was administered, with sufficient supplements (if necessary) to maintain an activated clotting time (ACT) ≥ 250 seconds during interventions.

After baseline collection of peripheral blood samples, patients were randomized to an intravenous infusion of GSH (2500 mg/25 ml of Glutathione Sodium Salt, Biomedica Foscama Group, Rome, Italy) or placebo (saline solution) over 10 min before p-PCI. The two solutions appeared identical in size and colour to ensure blinding. Study participants, investigators and the laboratory staff remained blinded until the statistical analysis was performed by an independent researcher who was not involved in the study.

Patients underwent p-PCI according to standard protocols. The use of thrombus aspiration, glycoprotein IIb/IIIa inhibition was left to the discretion of the treating physician. Multivessel PCI was performed in a staged fashion (7 to 10 days from index procedure).

All patients had drug-eluting stents implanted in treated vessels. After interventions, GSH was infused at the same doses at 24, 48, 72 hours elapsing time. Further blood samples were obtained at the end of procedure and 5 days from index procedure.

After 60'-90', a post-procedural 12 leads- ECG for ST measurement were performed.

Corrected TIMI frame count (cTFC) and TIMI myocardial perfusion grade (TMPG) were assessed after pPCI as previously described [21]. An external Core Lab processed the data (G.P and G.P: independent cardiologists). Digital angiograms were analyzed off-line with the use of an automated edge detection system (Cardiovascular Medical System, MEDIS Imaging Systems, Leiden, the Netherlands).

Randomization and blinding

An individual not involved in the study assigned codes (using a computer-generated random sequence) to the study treatment with a random allocation of patients to an intravenous infusion of GSH (2500 mg/25 ml over 10 min) or placebo (saline solution) before p-PCI. The interventional cardiologists who performed p-PCI, those who analyzed digital angiograms and the laboratory technicians were unaware of study treatment allocation.

Primary Endpoint

The primary endpoint was the change on oxidative stress markers levels after 2 hours from p-PCI in patients treated with GSH as compared with placebo.

Secondary Endpoints

The secondary endpoints included the assessment of: (i) changes of oxidative stress markers levels after 5 days from the p-PCI in patients received GSH or placebo; (ii) changes in serum cTpT, biochemical markers of myocardial cell damage, in patients received GSH or placebo before and after 5 days from the procedure.

Peripheral blood samples

Blood samples were drawn from antecubital vein, before the start of procedure and after stent deployment in all patients and then collected into tubes without anticoagulant or with 3.8% sodium citrate, lithium heparin and EDTA and centrifuged at 300×g for 10 min to obtain supernatant. All plasma and serum aliquots were stored at -80°C in appropriate cuvettes until assayed.

Markers of oxidative stress and antioxidant system (i.e. H_2O_2, H_2O_2 breakdown activity (HBA) and 8-iso-PGF2α) were analyzed in serum samples collected before p-PCI, 2 hours and 5 days after p-PCI. Due to the chemical properties of the oxidative stress markers, to avoid a long-time storage of blood samples and guarantee the laboratory test quality the analyses were performed within 6 months from the collection.

Serum cardiac Troponin T (cTpT) was measured at admission, before the procedure, 6 and 12 hours after reperfusion, and thereafter once a day up to 5 days. Serum cTpT levels were measured using ELISA Kit (Elabsciences).

H_2O_2 production

The H_2O_2 was evaluated by a Colorimetric Detection Kit (Arbor Assays, Ann Arbor, Michigan, US) and expressed as μM. Intra-assay and inter-assay coefficients of variation were 2.1% and 3.7%, respectively.

Determination of % HBA in peripheral serum

The evaluation of the ability to detoxify H_2O_2 was assessed by the analysis of **the HBA** in serum with HBA assay kit (Aurogene, Rome, Italy, code HPSA-50). The % of HBA was calculated according to the following formula: % of HBA = [(Ac-As) / Ac] X 100 where Ac is the absorbance of H_2O_2 1.4 mg/ml and As is the absorbance in the presence of the serum sample.

Serum Nitric Oxide (NO) bioavailability

A colorimetric assay kit (Cell Biolabs, San Diego, CA, US) was used to determine NO bioavailability by measurement of the nitric oxide metabolites nitrite and nitrate (NOx) in the serum. Intra-assay and inter-assay coefficients of variation were 2.9% and 1.7% respectively.

Serum 8-iso-Prostaglandin F2α formation

Concentration of 8-iso-PGF2α in serum was measured by validated enzyme immunoassay (EIA) method (DRG International, Springfield, NJ, USA). Intra-assay and inter-assay coefficients of variation were 5.8% and 5.0% respectively. Values were expressed as pmol/L.

Myocardial function

After 120 minutes and 5 days from the intervention, left ventricular end-diastolic volume (LVEDV), left ventricular end-systolic volume (LVESV) and ejection fraction (LVEF) were calculated by the biplane Simpson's rule, as recommended by the American Society of Echocardiography. The mean values of three measurements were used for statistical evaluation.

Sample Size Calculation

For the present preliminary analysis, the sample size calculation was estimated considering previous data available for 8-iso-PGF2α levels [22]. A sample size of 25 patients undergoing GSH infusion provided an intervention study with 80% power to detect a 20% reduction in plasmatic 8-iso-PGF2-α levels measured at the end of successfully reperfusion with respect to the placebo group. We also assumed a 25% SD in each group.

Statistical analysis

Categorical variables were reported as counts (percentage) and continuous variables as means ± standard deviation (SD). We tested the independence of categorical variables by $\chi 2$ test and the normal distribution of continuous variables by Kolmogorov-Smirnov test. We used Student paired and unpaired t test, repeated measure ANOVA and Pearson product-moment correlation analysis to evaluate normally distributed continuous variables. Appropriate nonparametric tests (Mann-Whitney U test, Wilcoxon rank test and Spearman rank correlation test) were employed for all the other variables. As an overall nonparametric ANOVA, the Friedman test for the analysis of intragroup variations was used. In cases of significance, we compared pair related samples using the Wilcoxon test. The intergroup analysis was performed with the nonparametric Mann–Whitney U-test. Only two-tailed probabilities were used for testing statistical significance. Probability values <

0.05 were regarded as statistically significant. All calculations were made with the computer program STATISTICA 7 (StatSoft, Tulsa, OK, USA).

Results

Twenty-five patients randomly received GSH and 25 placebo. All patients completed the phases of the study (Figure 1). All patients had a TIMI flow grade equal to 0 or 1 requiring percutaneous treatment. Clinical and angiographic characteristics of patients are shown in Table 1 and 2. The baseline characteristics were well balanced between the two groups. In both groups, neither side effects during the infusion, nor adverse events during the short observation period were recorded.

Oxidative stress, antioxidant status and vascular function in peripheral samples. Biochemical data are summarized in Table 3. Baseline H_2O_2 and 8-iso-PGF2α levels were similar between treated patients and controls. After PCI, a significant reduction of H_2O_2 production and 8-iso-PGF2α levels was observed in GSH group as compared to controls (Figure 2A and 2B). Moreover, a significant increase in HBA and NO bioavailability was observed (Figure 2C and 2D).

At the 5 days from index procedure, a persistent significant reduction of H_2O_2 production and a sustained increase in HBA and NO bioavailability was observed in the GSH group as compared with controls (Figure 2A-D).

Serological markers of myocardial injury. Baseline cTpT mean values were similar between GSH and placebo groups (176.0 ± 20.9 pg/ml vs. 165.4± 20.9 pg/ml, p=0.079). At 6 hours, no changes in cTpT values were found in GSH-treated patients (172.1±27.7 pg/ml vs. baseline, p=0.065). At 12 hours and 5 days after pPCI, GSH-treated patients showed a progressive decrease of cTpT levels (170.0 ± 44.7 pg/ml and 137.9 ± 23.7 pg/ml; -21±23.1%, p=0.009 vs. baseline). Differently, a significant increase and persistence of high values of cTpT were observed in placebo group (T6, 169.9±16.3 pg/ml, T12, 183.0 ± 34.8 pg/ml and T5d, 181.9 ± 18.0 pg/ml; +12.4±23.1%, p=0.029 vs. baseline) (Figure 3A). A modest correlation between percentage changes of H_2O_2 and cTpT levels from baseline to 5 days was found in treated group (Figure 3B).

Myocardial Reperfusion indexes. Post-procedural cTFC values did not show a statistically significant reduction between treated and control groups (20.7±7.3 vs. 23.4±5.1, p=0.156). Interestingly, 6 patients (24%) in the placebo group and 15 (60%) patients in GSH group reached lower-risk (<=20 frames/s) cTFC class (p=0.019). After PCI, TMPG ≥ 2 was assessed in 21 patients (84%) and 14 patients (56%) of the GSH and placebo groups, respectively (p = 0.064). Of note, 11 patients (44%) of the GSH group only had TMPG=3 (p=0.0002 vs. controls). Post-reperfusion cTFC values showed a significant correlation with changes of 8-iso-PGF2α (R=0.55, p=0.012) levels from baseline.

Myocardial function. Myocardial function was not different between groups after either baseline or at discharge. There was no significant difference between groups regarding LVEF, LVEDV or LVESV at any time point (Table 4).

Discussion

This pilot study demonstrates that in the setting of STEMI reperfusion the rapid onset and prolonged antioxidant (scavenging) activity obtained by infusion of GSH before and after primary PCI reduces the oxidative stress markers. The improvement of the antioxidant status resulted in a significant decrease of cardiac troponin, marker of myocardial damage.

Data from experimental and clinical studies suggest that following reperfusion myocardial cells death largely contributes to the final infarct size.[23,24] On the other hand, the extent of damaged myocardium is the most important predictor of adverse ventricular remodeling and it is linearly dependent upon the amount of myocardial salvage by and after reperfusion. Thus, attenuation of pro-oxidant state is an important goal in cardioprotective interventions.[25] Noteworthy, the serum of GSH treated patients showed a greater capacity to detoxify H_2O_2 evaluated by the HBA, an assay that measure the percentage of H_2O_2 neutralized into the samples.[26] We found an early and considerable increase of HBA, with positive effects on myocardial cell survival**,** assessed by cTpT.

Current evidences demonstrate that oxidant environment promotes cardiomyocyte death in the first few minutes of reflow suggesting the existence of a tight window of effective cardio-protection.[27,28] Therefore, ROS-induced injury may continue for weeks to months as a result of activation of programmed cell death. Our data have shown a persistent heightened oxidative status along with decreased scavenging activity in untreated patients. This behavior makes the duration of pharmacologic interventions a central point of cardio-protection strategies. In the present study, GSH infusion, starting just before reperfusion with subsequent administration up to 3 days after, promoted early and sustained increase of serum HBA with attenuated production of H_2O_2 which was highly related to progressive significant reduction of serological signs of myocardial injury. In addition, our data show a progressive significant decrease of serum cTpT release during the 5 days of reperfusion in the GSH-treated patients compared with the control group resulting in a 21% reduction of myocardial damage. Despite that, in our population, the systolic function was not different between groups after reperfusion, although a trend towards reduced LVEDV was observed in treated patients. A possible explanation relies on the fact that inside the area at risk variable amount of hibernated and stunned myocardium may coexist, thus affecting the prompt recovery of contractility after reperfusion.[29]

Cells have a number of mechanisms for dealing with the toxic effects of oxygen. One of the most important is connected with the widely distributed tripeptide thiol glutathione.[16,30] In particular, the glutathione redox cycle is a more efficient antioxidant protective mechanism of the heart, which acts by maintaining thiol groups of enzymes and other proteins in their reduced state thus preventing cell membrane lipid peroxidation and limiting cardiomyocytes loss.[31] Furthermore, in our study, a close relation between reduced myocardial reperfusion and increased of 8-iso-PGF2α serum levels has been observed, suggesting that oxidative unbalance may be involved in microcirculation functional damage. As previously reported, impaired tissue-level perfusion develops within minutes of established acute revascularization of ischemic areas[32] and persists for at least 1 week.[33] In this context, there is robust evidence that ROS mediated

isoprostanes production contributes importantly to the post-reperfusion microvascular impairment.[22,34] Current findings implement this observation by demonstrating a sustained production of isoprostanes up to 5 days after reperfusion thereby suggesting their contributory role in the pathogenesis and persistence of microvascular dysfunction that may affect myocardial cell survival. The infusion of GSH before and 24, 48, 72 hours after pPCI reduced isoprostanes serum levels and their reduction was linked to improvement of myocardial reperfusion indexes. Moreover, the increase in extracellular peroxide oxidants may reduce bioavailability of nitric oxide that is thought to contribute to promoting platelet hyperactivity and vasoconstriction.[13] In our study, GSH supplementation seems to have a role in preserving NO bioavailability and its vasodilator capacity at microcirculatory level.

The Strengths and Limitations of the Study

The positive effects on reperfusion indexes and on biochemical signs of myocardial necrosis suggest the value of prophylactic and prolonged GSH administration in preventing reperfusion injury. Thus, in patients undergoing pPCI the infusion of a powerful antioxidant scavenger, such as GSH, may be useful to improve microcirculatory perfusion in order to further blunt the injury of myocardial cells.

Some limitations deserve to be discussed.

The small sample size of the study and the lack of morfologic assessment of both infarct size and microvascular obstruction extent between the two groups, actually, limit the clinical application of these findings. Within a defined area at risk, the manifestations of ischemia-reperfusion vascular injury go from reversible functional impairments to irreversible structural damage and contribute to final amount of infarct myocardium. In absence of morphologic imaging, the qualitative evaluation of GSH-induced improvement of myocardial reperfusion indexes, as assessed in our study, might only represent the effect of a preserved microcirculatory responsiveness to vasoactive substances (i.e. NO) but unable to limit the expansion of myocardial cell damage. Indeed, other mechanisms, such as interstitial edema and inflammatory reaction, which induce a sustained impairment of

microvascular perfusion, may primarily act to increase the amount of irreversible injured myocardium thus promoting adverse ventricular remodeling.

In conclusion, in this pilot study, we have shown that a short-term prophylactic GSH infusion mitigates the negative effects of the excessive and persistent H_2O_2 formation on myocardial cells. The findings of the present study require to be confirmed through an adequately powered STEMI population. A larger trial with a prolonged follow-up for evaluation of clinical endpoints is needed to confirm the role of GSH administration as cardioprotective therapy.

Figure Legend

Figure 1. CONSORT flowchart

Figure 2. H_2O_2 production (A), 8-iso-PGF2α formation (B), hydrogen peroxide breakdown activity (HBA) (C) and NO bioavailability (D) at baseline, after 2 hours (T2h) and at the 5 days (T5d) from the PCI in patients received GSH (n=25, dashed line) or placebo (n=25, continuous line). Data are expressed as mean±SEM (***p <0.001, **p <0.01, *p <0.05).

Figure 3. cTpT levels (A) at baseline, after 6 hours (T6h), 12 hours (T12h) and at the 5 days (T5d) from the PCI in patients received GSH (n=25, dashed line) or placebo (n=25, continuous line). Data are expressed as mean±SEM (***p<0.0001 vs. T0, *p<0.05 vs. T0, $^{\$}$p<0.05 between groups). Linear correlation between % Δ cTpT and % Δ H_2O_2 in GSH treated group (B).

Contributors

GTa, EM led on the conception, design and writing of the study with substantial contributions to the design, writing, critical review of intellectual content; GTr, AA, AP, NV, RM, enrollment of patients; RC, VC, CN, provided laboratory analyses; VR, SB, provided further essential statistical advice and expertise on the study protocol; FB, LL, MP, CG, providing expert clinical support; AG, MD, made substantial contributions to the trial design and management.

Ethics and dissemination

Italian Medicines Agency (AIFA) authorization and single Ethic Committee approval has been obtained for all the centers participating the study. Dissemination of results will be via peer-reviewed research publications both online and in print, conference presentations, posters, patient forums and Trust bulletins.

Data Availability Statement

The data set is available on request from the corresponding author.

Conflicts of Interest

Authors declare that there are not conflicts of interest.

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Table 1. Clinical characteristics of the study population

Variables	GSH group (n=25)	Placebo group (n=25)	P value
Age (y, mean±SD)	66 ± 10.7	66.9 ± 9.1	0.74
Male, n (%)	15 (60)	13 (52)	0.98
Body-mass index§ (mean+SD)	26.9 ± 3.9	20 ± 3.8	0.38
Killip class ≥ 3, n (%)	2 (8)	0 (0)	0.47
Diabetes Mellitus, n (%)	5 (20)	5 (20)	1
Hypertension, n (%)	14 (56)	17 (68)	0.56
Dyslypidemia, n (%)	11 (44)	13 (52)	0.77
Statin use, n (%)	8 (32)	8 (32)	1
Smokers, n (%)	17 (68)	13 (52)	0.38

§ The body-mass index is the weight in kilograms divided by the square of the height in meters.

Table 2. Angiographic parameters

Variables	GSH group (n=25)	Placebo group (n=25)	P value
Ischemia time# (min; mean ± SD)	286 ± 88	270 ± 96	0.85
Thrombus Burden ≥ 3, n (%)	12 (48)	11 (44)	0.77
Thrombus aspiration, n (%)	13 (52)	12 (48)	0.87
GP IIb/IIIa inhibitors, n (%)	2 (8)	3 (12)	0.63
MVD, n (%)	13 (52)	11 (44)	0.77
2 vessels,	8 (32)	5 (20)	
3 vessels,	5 (20)	6 (24)	
Staged PCI, n (%)	9 (36)	5 (20)	0.89
IRA:			
LAD, n (%)	10 (40)	9 (36)	0.77
LCx, n (%)	5 (20)	6 (24)	0.73
RCA, n (%)	10 (40)	10 (40)	1

PCI = percutaneous coronary intervention; **IRA** = infarct related coronary artery; **LAD** = left anterior descending coronary artery**; LCx** = left circumflex coronary artery; **RCA** = Right coronary artery. #ischemia time was defined as the timing between symptom onset and balloon inflation.

Table 3. Biochemical data

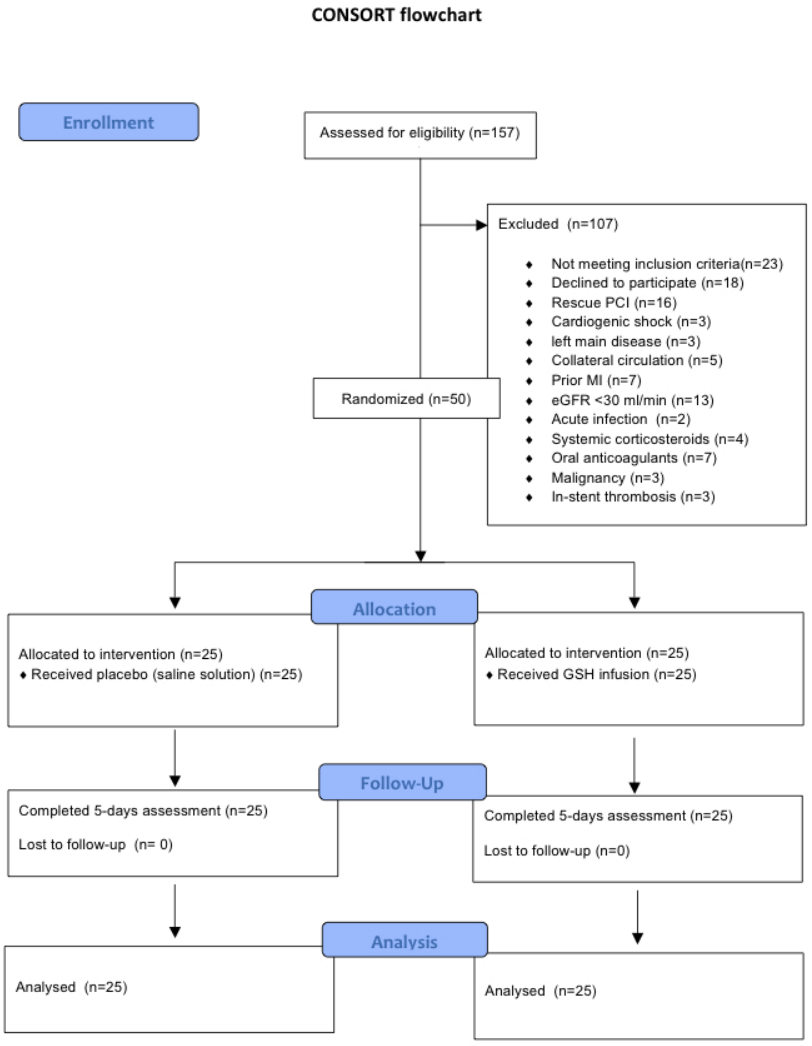
	Baseline			Reperfusion 2h			Follow-up (5 days)		
Variable	**GSH**	**Placebo**	**p**	**GSH**	**Placebo**	**p**	**GSH**	**Placebo**	**p**
H_2O_2 µM, mean±SD	40.6±8.4	43.6±11.6	0.305	28.4±12	42.8±14.1	0.0003	24±7	39.5±17.3	0.0001
Δ				-12.1±15.2	-0.7±17.9	0.03	-16.6±11.0	-4.1±20.14	0.009
8-iso-PGF2α pmol/L, mean±SD	214.6±81.1	211.9±92.1	0.91	163.6±44.7	217.6±51.6	0.0003	159.9±34.2	213.1±50.9	0.0001
Δ				-50.9±92.9	-3.3±1.29	0.02	-54.6±62.1	-1.2±115.7	0.02
HBA %, mean±SD	43.6±7.4	43.4±11.9	0.94	57.9±8.6	43.9±8.7	0.0001	62.9±10.5	45.2±13.0	0.0001
Δ				+14.9±5.5	+0.4±14.9	0.0004	+19.4±10.2	+1.8±17.1	0.0001
NO µM, mean±SD	16.3±5.7	16.5±4.7	0.89	27.7±7,2	22.4±10	0.0356	35.5±8.1	23.5±15.5	0.0013
Δ				+11.4±6.8	+5.8±10.5	0.05	+19.2±9.7	+7.0±14.7	0.002

GSH = reduced Glutathione; **H_2O_2** = Hydrogen Peroxide; **8-iso-PGF2α** = 8-iso-Prostaglandin-F2α; **HBA** = H_2O_2 break-down activity

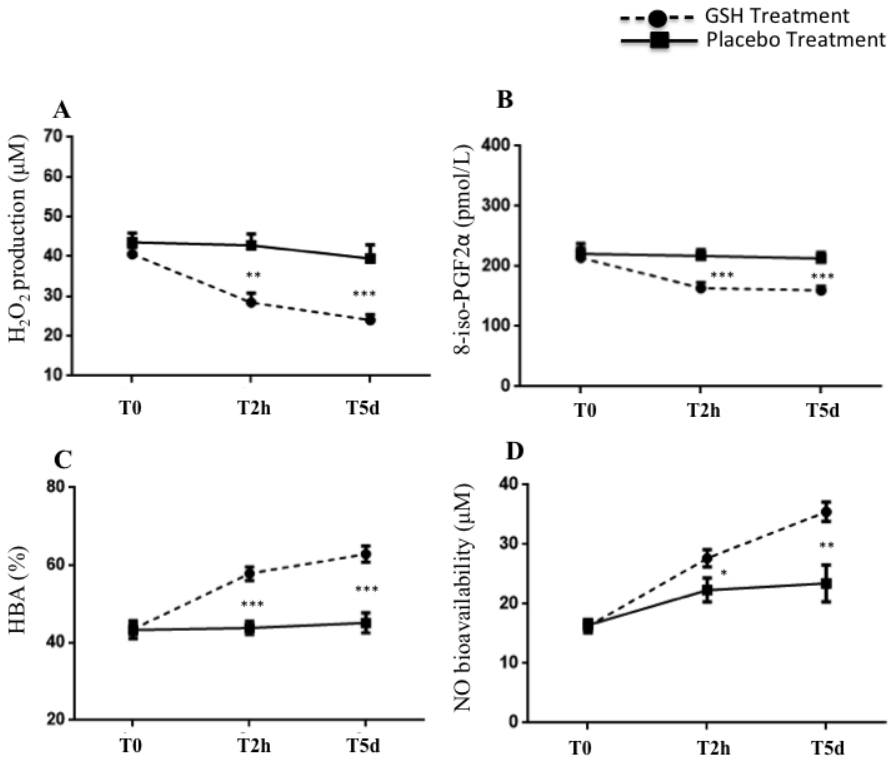
Table 4. Left Ventricular echocardiographic parameters at baseline and at follow-up

Echo parameters	Placebo (n= 25)	GSH (n=25)	*P*-value
Baseline			
LVEDV (mL/m2)	121.3 ± 17.2	124.4 ± 22.3	0.44
LVESV (mL/m2)	65.4 ± 11.3	66.3± 13.2	0.91
LVEF (%)	47.5 ± 4.9	46.9 ± 4.8	0.42
Follow-up			
LVEDV (mL/m2)	118.1 ± 17.8	113.2 ± 14.1	0.42
LVESV (mL/m2)	60.9 ± 10.7	58.8± 12.5	0.91
LVEF (%)	49.1 ± 3.2	49.8 ± 3.7	0.42

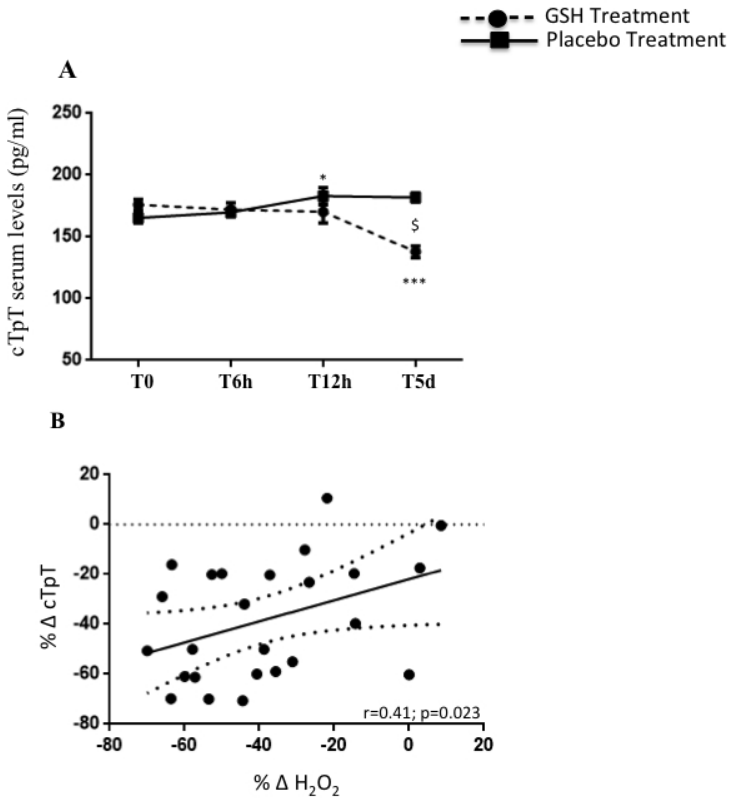
LVEDV = left ventricular end-diastolic volume; **LVEDSV** = left ventricular end-systolic volume; **LVEF** = left ventricular ejection fraction-diastolic volume



254x338mm (72 x 72 DPI)



254x338mm (72 x 72 DPI)



254x338mm (72 x 72 DPI)

EudraCT Number: 2014-004486-25
Sponsor's Protocol Code Number: GSH2014
National Competent Authority: Italy - Italian Medicines Agency
Clinical Trial Type: EEA CTA
Trial Status: submitted data of the pilot study. The trial is ongoing.
Date on which this record was first entered in the EudraCT database: 2014-12-04
Link: https://www.clinicaltrialsregister.eu/ctr-search/trial/2014-004486-25/IT/

A. Protocol Information
Member State Concerned: Italy - Italian Medicines Agency
EudraCT number: 2014-004486-25
Full title of the trial: Prevention of the reperfusion myocardical damage in patients with acute myocardial infarct (STEMI) submitted to primary PCI through infusion of intravenous glutathione.
Sponsor's protocol code number: GSH2014

B. Sponsor Information
Sponsor 1: University Hospital "Policlinico Umberto I"
Name of organization providing support: University Hospital "Policlinico Umberto I", Rome, Italy.
Functional name of contact point: Enrico Mangieri, University Hospital "Policlinico Umberto I".
Viale del Policlinico, 155 – Rome, Post code: 00161, Italy
E-mail: enrico.mangieri@uniroma1.it

D. IMP Identification
IMP to be used in the trial has a marketing authorisation: Yes
Trade name: TAD
Name of the Marketing Authorisation holder: Biomedica Foscama Group S.p.A.
Country which granted the Marketing Authorisation: Italy
Pharmaceutical form: Powder and solvent for solution for infusion
Routes of administration for this IMP: Intravenous use
Information on Placebo
Pharmaceutical form of the placebo: saline solution
Route of administration of the placebo: Intravenous use

E. General Information on the Trial
Medical condition or disease under investigation
Medical condition(s) being investigated: ST-Segment Elevation Myocardial Infarction (STEMI).
Medical condition in easily understood language: acute myocardial infarct
Therapeutic area: Diseases [C] - Cardiovascular Diseases [C14]
Objective of the trial
Main objective of the trial: To verify if the intravenous infusion of "Glutathione Sodium Salt" it is able to reduce the level of oxidative state in the area of myocardial infarction.
Secondary objectives of the trial: To verify if the intravenous infusion of "Glutathione Sodium Salt" during the procedures of primary PCI it is able to limit the extension of the ischemic area, to reduce the incidence of the no-reflow, to improve the degree of myocardial blush and to decrease the indexes of suffering post-procedural ischemia (ST elevation; release of myocardial necrosis markers).
Principal inclusion criteria: STEMI patients submitted to p-PCI up to 12 hours.
Age≥18 years. Women and Men. Signed informed consent

Principal exclusion criteria:
Patients with cardiac arrest, ventricular fibrillation, cardiogenic shock, stent thrombosis, previous acute myocardial infarction, or angina within 48 hours before infarction were not included in the study. Patients with evidence of coronary collaterals (2-3 Rentrop) to the region at risk on initial coronary angiography (at the time of admission) will be excluded. Moreover, patients with EF ≤30%, impaired renal function (creatinine > 3.0 mg/dl), recipient of heart transplant, a life expectancy less than 12 months, has known allergies to aspirin, clopidogrel bisulfate, heparin, contrast media or stainless steel that cannot be managed medically were excluded.
Patient needs therapy with warfarin or currently participating in an investigational drug or another device study were not considered enrolling.

End points
Primary end point(s): The primary endopoint will consist in the assessment of the effects of the infusion of "Glutathione Sodium Salt" on the reduction of the oxidative markers and inflammation after PCI.
Timepoint(s) of clinical evaluation of this end point: before, 2 hour and 5 days from the p-PCI

Secondary end point(s): The secondary endpoint will include: (1) the assessment of the variations of the corrected TIMI frame count (cTFC) and the TIMI Myocardial Perfusion Grade (TMPG) after p-PCI; (2) the assessment of the middle values of peak of the cardiac Troponin, after the procedure; (3) to verify, through telephone contact or a programmed visit, the principal adverse clinical events as death, acute myocardial infarct, stent's thrombosis of the treated vessels or the occurrence of a new revascularization, up to 6 months after the procedure.
Medical Doctors don't have the knowledge both about the possible infusion of the Glutathione Sodium Salt, in the examined patient, then others clinical data.

Moreover, serological levels of Troponin and creatinine will be measured before the p-PCI and after the procedure (2, 6, 12 and 24 hours).
Besides, through 2D Echocardiography with Simpson's biplane method the FE will be calculate at admission and after hospital discharge.
If clinical-instrumental signs of ischemia will rise up, the patient will be submitted to a new angiography.

Definition of the end of the trial and justification where it is not the last visit of the last subject undergoing the trial: LVLS or telephonic contact
Population of Trial Subjects
Trial has subjects under 18: No
Adults (18-64 years): Yes
Number of subjects for this age range: 30
Elderly (>=65 years): Yes
Number of subjects for this age range: 60
Female: Yes
Male: Yes
Patients: Yes
Specific vulnerable populations: Yes
Women of childbearing potential not using contraception: Yes
Women of child-bearing potential using contraception: Yes

Pregnant women: No
Nursing women: No
Emergency situation: No
Subjects incapable of giving consent personally: No
Planned number of subjects to be included: 90

F. Investigator Networks to be involved in the Trial

N. Review by the Competent Authority or Ethics Committee in the country concerned
N. Competent Authority Decision: Authorised
N. Date of Competent Authority Decision: 2015-01-13
N. Ethics Committee Opinion of the trial application: Favourable
N. Date of Ethics Committee Opinion: 2015-02-12
N. Centers involved in the study: Department of Heart and Great Vessel "A. Reale", Sapienza University of Rome (coordinator centre) - "Santa Maria" Terni Hospital - "San Giovanni Evangelista" Tivoli Hospital, all in Italy.
P. End of Trial Status: analyzed as pilot study the first 50 enrolled patients. Ongoing.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	2
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4
	2b	Specific objectives or hypotheses	5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	6
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	NA
Participants	4a	Eligibility criteria for participants	6
	4b	Settings and locations where the data were collected	7
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	7
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	7-8
	6b	Any changes to trial outcomes after the trial commenced, with reasons	NA
Sample size	7a	How sample size was determined	9
	7b	When applicable, explanation of any interim analyses and stopping guidelines	9
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	7
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	7
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	7
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	7
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those	7

		assessing outcomes) and how	
	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	9-10
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	NA
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	7
	13b	For each group, losses and exclusions after randomisation, together with reasons	7
Recruitment	14a	Dates defining the periods of recruitment and follow-up	6
	14b	Why the trial ended or was stopped	NA
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	21-22
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-11
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	NA
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	NA
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	10
Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	13-14
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	14
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	14
Other information			
Registration	23	Registration number and name of trial registry	5
Protocol	24	Where the full trial protocol can be accessed, if available	Supplementary file
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	16

*We strongly recommend reading this statement in conjunction with the CONSORT 2010 Explanation and Elaboration for important clarifications on all the items. If relevant, we also recommend reading CONSORT extensions for cluster randomised trials, non-inferiority and equivalence trials, non-pharmacological treatments, herbal interventions, and pragmatic trials. Additional extensions are forthcoming: for those and for up to date references relevant to this checklist, see www.consort-statement.org.