Thiol-based cardioprotection

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CHAPTER 7

Sodium Thiosulfate in Acute Myocardial Infarction: a Randomized Clinical Trial

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ABSTRACT

Background
Experimental evidence shows that sodium thiosulfate (STS), a potent antioxidant and hydrogen sulfide (H_2S) donor, attenuates reperfusion injury.

Objectives
In this proof-of-principle trial, we aimed to determine whether STS administration at time of percutaneous coronary intervention reduces myocardial infarct size.

Methods
In a randomized, double-blind trial, we assigned patients presenting with ST-segment elevation myocardial infarction (STEMI) to receive either 12.5 grams of STS intravenously or matching placebo immediately at arrival at the hospital and a second dose 6 hours later. The primary outcome was infarct size as assessed by cardiac magnetic resonance imaging after 4 months. Secondary outcomes were the release of creatine kinase-MB (CK-MB), left ventricular ejection fraction (LVEF), and N-terminal pro-B-type natriuretic peptide (NT-proBNP) concentration. Safety was also assessed.

Results
We studied 373 patients; 186 assigned to the STS group and 187 to the placebo group. The mean infarct size at 4 months was 8.0% (standard deviation 7.0) of the left ventricle in the STS group as compared with 8.9% (standard deviation 7.4) in the placebo group (P=0.55). The peak CK-MB, LVEF and NT-proBNP values were also comparable between groups. The combined incidence of cardiovascular death, re-infarction and unplanned revascularization at 4 months was 6 (3.2%) in STS group as compared to 11 (5.9%) in the placebo group (P=0.22). Nausea and vomiting occurred more frequent in the STS group.

Conclusions
In our proof-of-principle trial, administration of STS at time of reperfusion was not associated with smaller infarct size compared to placebo.

INTRODUCTION

Myocardial infarction is a common cause of disability and death worldwide.[1] Infarct size is the major determinant for the future development of heart failure and reduced life expectancy.[2] Major progress has been made to limit infarct size, mainly by thrombolysis and primary percutaneous coronary intervention (PCI) in combination with antiplatelet therapy. Despite this progress, death rate, heart failure and recurrent cardiac events continue to remain substantial in patients presenting with acute myocardial infarction.[3,4]

Reperfusion of ischemic myocardium can paradoxically induce myocardial injury and experimental data suggest that this injury can contribute up to 50% of the final myocardial infarct size.[5–9] Myocardial reperfusion injury is a therapeutic target for which currently no effective treatment exists and the search for an effective therapy is ongoing.[8,10–12] Sodium thiosulfate (STS) is a strong antioxidant and anti-inflammatory compound with vasoactive properties.[13] Moreover, as donor of the physiological gaseous signaling molecule hydrogen sulfide (H_2S) it might also exert additional H_2S-related beneficial effects, including the maintenance of mitochondrial integrity, vasodilatation, activation of antiapoptotic and antioxidant pathways, and anti-inflammatory effects.[14–17] STS is clinically used for the treatment of acute cyanide poisoning, calciphylaxis, carbon monoxide toxicity, and cisplatin toxicities in cancer therapy.[18–21] H_2S donating compounds, including STS, have also been successful in reducing reperfusion injury in a wide variety of preclinical models.[13,22–26] In an earlier dose-escalation pilot study we demonstrated that STS was well tolerated in patients presenting with an acute coronary syndrome undergoing PCI.[27]

In this proof-of-principle trial, we tested the hypothesis that STS treatment reduces infarct size in patients presenting with ST-segment elevation myocardial infarction (STEMI).

METHODS

Trial design and oversight

The Groningen Intervention Study for the Preservation of Cardiac Function with STS after STEMI (GIPS-IV) trial is an investigator-driven, randomized, controlled, double blind trial conducted in 3 high-volume PCI centers in the Netherlands: University Medical Centre Groningen (UMCG), Groningen, University Medical Centre Utrecht (UMCU), Utrecht, and Treant hospital, location Emmen. Details of the trial design have been previously published.[28] All study procedures were in accordance with the declaration of Helsinki and Good Clinical Practice guidelines. The conduct of the trial was supervised by the trial steering committee. An independent data and safety monitoring board (DSMB) oversaw the safety of the trial. The authors designed and coordinated the trial, oversaw the study conduct and reporting, managed the database, and wrote all drafts of the manuscript. All the authors vouch for the accuracy and completeness of the reported data and analyses. The contents of this article

are consistent with the research protocol, which was approved by the ethics committee (Ref. 2016.381; Groningen, the Netherlands) and national authority. Before commencing enrollment, the trial was registered in a clinical trial registry under number NCT02899364 (www.clinicaltrials.gov). Detailed information about the trial organization is available in **Supplementary methods I**.

Study population

Adults who presented with a first STEMI were eligible if their complaints started within 12 hours before presentation and their symptoms were ongoing and/or ST-segment elevation was persistent upon arrival at the cardiac catheterization laboratory. Patients with a history of prior myocardial infarction, coronary artery bypass grafting or cardiomyopathy, a malignancy treated with chemo- and/or radiotherapy (chest region), or any condition which did not allow the patient to successfully undergo cardiac magnetic resonance imaging (CMR) or participate in the study were excluded. Details of the inclusion and exclusion criteria are provided in the trial protocol.

Trial procedures

The study procedures were designed not to delay primary PCI. Immediately after arrival at the cardiac catheterization laboratory, witnessed oral consent was obtained by the interventional cardiologist and patients directly received the next-in-sequence randomized kit of either STS 12.5 grams or matching placebo. Study medication was dissolved in 250mL of normal saline and administered intravenously (i.v.) in 20-30 minutes before and during PCI. Six hours after the first dose, a second dose of study medication was administered. The rationale behind the timing and dosage of study medication has been previously published.[28] Known side effects of STS include nausea, vomiting and hypotension,[20] which were specifically monitored before and after each dose of study medication. Due to high incidences of nausea and vomiting observed during the execution of the trial the DSMB recommended to preventively administer antiemetics (metoclopramide 10mg i.v.) before each dose of study medication. The trial protocol was amended accordingly. During hospitalization, written informed consent was obtained and creatine kinases MB (CK-MB) were measured to determine enzymatic infarct size. Four months after randomization, participants were scheduled for a hospital visit to obtain CMR and to assess adverse events and N-terminal pro-B-type natriuretic peptide (NT-proBNP). In case a participant declined CMR, adverse events were assessed by telephone. Study medication was produced, randomized, and labelled according to Good Manufacturing Practice by A15 Pharmacy (Gorinchem, the Netherlands). Randomization was performed in a 1:1 ratio in permuted blocks of 4, with stratification by recruiting site and for anterior versus non-anterior myocardial infarction. The patient, interventional cardiologist, all caregivers, data collectors, and the CMR core laboratory were blinded to treatment allocation.

Trial outcomes

CMR is the preferred method for the identification of potential benefits associated with new cardioprotective strategies.[29] The primary outcome of this trial was infarct size expressed as percentage of LV mass. Infarct size was measured 4 months after randomization, a time in which the infarct healing is expected to be completed,[30] and infarct size no longer changes substantially.[31–33] Secondary outcome parameters included the effect of STS on peak CK-MB during index hospitalization, Left Ventricular Ejection Fraction (LVEF) on CMR at 4 months, and NT-proBNP concentration at 4 months follow-up. Clinical events were also assessed up to 4 months after randomization and included all-cause mortality, the combined incidence of Major Adverse Cardiovascular Events (MACE; cardiovascular death, re-infarction, unscheduled re-intervention), stent thrombosis, stroke, Implantable Cardioverter Defibrillator (ICD) implantation and hospitalization for chest pain or heart failure. All potential clinical endpoints were adjudicated by an independent endpoint adjudication committee blinded to treatment allocation. The endpoint definitions are available in **Supplementary methods II**.

CMR protocol

All CMR studies were performed on a 3T clinical MR scanner (multivendor Siemens, Philips), using a phased array cardiac receiver coil. Electrocardiogram-gated balanced steady-state free precession cine images were acquired during repeated breath holds in the standard long-axis views (4-, 3-, and 2-chamber view) and contiguous short-axis slices covering the entire left ventricle. Using identical slice locations, late gadolinium enhanced images were acquired at least 10 minutes after intravenous administration of a gadolinium-based contrast agent (0.2 mmol/kg) with a single shot inversion recovery gradient-echo pulse sequence. The epi- and endocardial borders were outlined in end-systolic and end-diastolic images to measure left ventricular volumes and calculate LVEF. Infarct size was quantified using an automated method (full width at half maximum) with manual correction.[34,35] All CMR scans were evaluated by an independent core laboratory (Radboud UMC, Nijmegen, the Netherlands) using dedicated software (QMass, Medis Suite 3.2.28.0, Leiden, the Netherlands). The core laboratory was blinded to treatment allocation and clinical patient data. All CMR measurements and calculations were performed, and data was locked before unblinding.

Statistical analysis

This trial was designed as a proof-of-concept study. We considered a relative reduction of 33% in infarct size relevant.[33] In the previous GIPS-III trial the mean infarct size was 9.0% ± 7.9.[36] In order to have a 85% power to detect a 3% absolute difference in change in infarct size between the STS and placebo group, we calculated that 125 patients would need to be enrolled in each group, assuming a 2-sided α of .05. Based on experience from local and previous studies, we anticipated for a 33% dropout for the primary endpoint due to contraindications for CMR – for example ICD-implantation, claustrophobia or unable to obtain sufficient image quality for infarct size detection.[36–39] Therefore, we expected to need to include 380 patients to obtain a reliable primary outcome measure in 250 patients. Due to the covid-19 pandemic, the actual

drop-out for the CMR visit after 4 months was higher than anticipated, but this did not result in the recommendation of the DSMB or ethical committee to extend enrollment.

Baseline characteristics were summarized as mean ± standard deviation (SD) or median and interquartile range [IQR] depending on data distribution. Categorical variables were displayed as count and percentages (%). All analyses were performed according to a pre-specified analysis plan which was finalized before database lock and unblinding. No formal interim analysis took place. Missing data was not imputed. The primary outcome, infarct size, was analyzed with Beta regression on an intention-to-treat (ITT) basis. Treatment allocation, recruiting site and anterior myocardial infarction were added to the model as fixed variables. The regression coefficient for treatment allocation is the primary outcome and is reported as the (marginal average) difference in infarct size between the STS and placebo group, together with a *P*-value and 95% confidence interval. Likewise, a Beta regression within the per-protocol population was performed, including all patients that received complete treatment with study medication, without major protocol deviations. Irrespective of the primary outcome reaching statistical significance, pre-specified subgroup analyses were performed using regression analyses with a test for interaction for age (below vs above the median), gender, TIMI-flow pre-PCI (≤1 vs >1), infarct location (anterior vs. non-anterior myocardial infarction), ischemic time (below vs above the median), single vs multivessel disease and the time from start of study medication to first coronary intervention (below vs above the median). For analyses of secondary outcomes, when binary, treatment comparisons were performed using Fisher exact probability tests or Chi-square analysis. For continuous outcomes, independent samples T-tests or a Mann-Whitney U tests were used, as appropriate. A 2-sided α of 0.05 was considered statistically significant. Analyses were performed with STATA version 14.0 (STATAcorp, College Station, TX, USA).

RESULTS

From July 16th 2018 through March 2nd 2021, a total of 1,650 patients presenting with suspected STEMI in one of the three recruiting centers were screened for eligibility (**Supplementary Figure 1**). A total of 380 patients underwent randomization and received a first dose of study medication. Seven patients withdrew informed consent resulting in a study population of 373 participants. 186 participants were assigned to the STS group and 187 to the placebo group. The characteristics of the patients were well balanced in the two treatment groups at baseline (**Table 1**, **Table 2**) and at discharge (**Supplementary Table 1**). The mean age in the overall population was 62 years (SD 12), and 23.1% of the patients were women. The median time from onset of complaints to wire passage was 141 minutes (IQR 102-177). Prior to primary PCI, 371 (99.5%) patients received aspirin and all patients received a loading dose of a P2Y12 inhibitors and heparin. TIMI 0 or 1 flow prior to PCI was observed in 245 (65.7%) patients and the left anterior descending artery was identified as the culprit lesion in 152 (40.8%). After PCI a TIMI 0 or 1 flow was observed in 14 (3.8%) patients.

Table 1 | Characteristics of the Patients at Baseline

Characteristic	STS n =186	Placebo n =187
Demography		
Age at randomization (years), mean (SD)	62.3 (11.5)	61.8 (12.0)
Male sex, n (%)	140 (75.3%)	147 (78.6%)
Body Mass Index (kg/m^2), mean (SD)	27.3 (4.0)	27.1 (4.6)
Caucasian ethnicity, n (%)	181 (97.3%)	182 (97.3%)
Prior conditions, n (%)		
Hypertension	86 (46.2%)	82 (43.9%)
Dyslipidemia	66 (35.5%)	67 (35.8%)
Current smokers	73 (39.2%)	71 (38.0%)
Positive family history	79 (42.7%)	80 (42.8%)
Diabetes Mellitus	23 (12.4%)	28 (15.0%)
Previous MI	5 (2.7%)	1 (0.5%)
Previous PCI	4 (2.2%)	1 (0.5%)
Clinical characteristics		
Systolic blood pressure, mmHg, mean (SD)	138 (25)	143 (26)
Diastolic blood pressure, mmHg, mean (SD)	84 (17)	86 (16)
Heart rate, bpm, mean (SD)	73 (16)	75 (17)
Killip class I, n (%)	171 (96.6%)	180 (96.8%)
Laboratory parameters, median [IQR]		
Hemoglobin (mmol/L)	8.6 [8.1-9.2]	8.7 [8.0-9.3]
Creatinine (µmol/L)	75 [65-86]	75 [64-86]
CK (U/L)	127 [82-211]	134 [90-232]
CK-MB activity (U/L)	15 [12-20]	16 [13-23]
NT-proBNP (ng/L)	106 [40-221]	87 [43-216]
Glucose (mmol/L)	5.7 [5.5-6.1]	5.6 [5.5-6.1]

Baseline characteristics stratified by treatment allocation. Abbreviations: bpm, beats per minute; CK, creatine kinase; CK-MB, creatine kinase-myocardial band; IQR, interquartile range; MI, myocardial infarction; NT-proBNP, N-terminal pro B-type natriuretic peptide; PCI, percutaneous coronary intervention; SD, standard deviation; STS, sodium thiosulfate.

Table 2 | Procedural Characteristics

Characteristic	STS n =186	Placebo n =187
Time from symptom onset to start study medication, (min), median [IQR]	119 [80-193]	131 [90-215]
Time from symptom onset to wire passage, (min), median [IQR]	133 [97-203]	147 [104-233]
Single vessel disease, n (%)	103 (55.4%)	91 (48.7%)
Culprit territory, n (%)		
Left anterior descending	76 (40.9%)	76 (40.6%)
Circumflex or marginal	29 (15.6%)	27 (14.4%)
Right coronary artery	77 (41.4%)	77 (41.2%)
Left main	0 (0.0%)	1 (0.5%)
No clear culprit	4 (2.2%)	6 (3.2%)
Medication from first medical care to PCI, n (%)		
Aspirin	186 (100%)	185 (98.9%)
Loading dose of P2Y12 inhibitor	186 (100%)	187 (100%)
Heparin	186 (100%)	187 (100%)
Glycoprotein IIb/IIIa inhibitor	29 (15.6%)	33 (17.6%)
TIMI flow grade pre-PCI, n (%)		
0	114 (61.3%)	111 (59.4%)
1	10 (5.4%)	10 (5.3%)
2	29 (15.6%)	22 (11.8%)
3	32 (17.2%)	43 (23.0%)
Can not be defined	1 (0.5%)	1 (0.5%)
Proximal lesion, n (%)	77 (41.4%)	77 (41.2%)
Initial intervention of culprit lesion, n (%)		
PCI	181 (97.3%)	176 (94.1%)
CABG	1 (0.5%)	3 (1.6%)
Conservative	4 (2.2%)	8 (4.3%)
No reflow observed on angiography, n (%)	5 (2.8%)	7 (4.0%)
Distal embolization after PCI, n (%)	16 (8.8%)	10 (5.7%)
TIMI flow grade post-PCI, n (%)		
0	5 (2.8%)	4 (2.3%)
1	4 (2.2%)	1 (0.6%)
2	3 (1.7%)	10 (5.7%)
3	169 (93.4%)	161 (91.5%)

Abbreviations: CABG, coronary artery bypass graft; IQR, interquartile range; PCI, percutaneous coronary intervention; STS, sodium thiosulfate; TIMI, Thrombolysis in Myocardial Infarction.

Primary outcome

CMR was completed in 238 patients 4 months after randomization (median 4.0 months [IQR 3.8 to 4.5 months], full range 3.4 to 8.2 months). The primary outcome parameter, infarct size on CMR, could be determined in 116 patients in the STS group and 110 in the placebo group (**Supplementary Figure 1**). The baseline, procedural and discharge characteristics were also well balanced between the STS and placebo treated patients of the CMR population (**Supplementary Table 2**, **3** and **4**). Infarct size at 4 months after randomization did not differ between the STS and placebo treated patients. Mean infarct size in the STS group was 8.0% (SD 7.0) and 8.9% (SD 7.4) in the placebo group. The marginal average change in infarct size in the STS group was -0.6%, 95% confidence interval: -2.4% to 1.2%, *P*=0.55, compared to participants treated with placebo (**Figure 1**, **Table 3**).

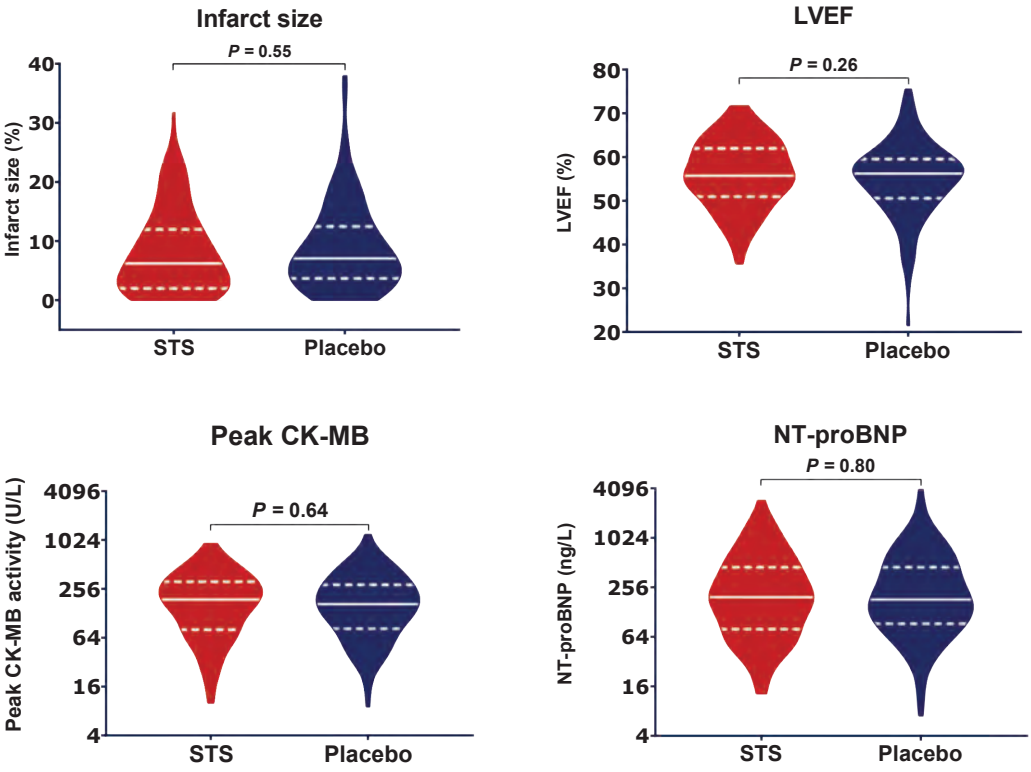


Figure 1 | Primary and secondary outcomes by allocated treatment

Violin plots showing medians (solid line) and interquartile ranges (dashed line) for the primary outcome, infarct size at 4 months follow-up, and secondary outcomes in patients treated with STS (red) and patients treated with placebo (blue). No significant differences were observed between treatment arms, suggesting no clinical benefit of STS in this relatively low risk study population. Abbreviations; CK-MB, Creatine Kinase-Myocardial Band; LVEF, left ventricular ejection fraction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; STS, sodium thiosulfate.

Secondary outcomes

Enzymatic infarct size was available for 248 patients; 124 in the STS and 124 in the placebo group. Missing values were due to early transfer to referring hospital or hemolytic sample. The median peak creatine kinase MB value was 191 U/L (interquartile range 81-315) in the STS group compared to 168 U/L (interquartile range 84-289) in the placebo group (*P*=0.64) (**Table 3**). LVEF at 4 months follow-up was determined by CMR in 230 patients. The average LVEF was 56.1% (SD 7.6) in the STS group compared to 54.9% (SD 8.7) in the placebo group (*P*=0.26). NT-proBNP at 4 months was also comparable for both treatment strata (**Table 3**).

Table 3 | Outcomes and clinical events at 4 months

Outcome	STS	Placebo	*P*-value
Primary outcome			
Infarct size (% of LV mass), mean (SD)	8.0 (7.0)	8.9 (7.4)	0.55 [a]
Secondary outcomes			
Peak CK-MB (U/L), median [IQR] [b]	191 [81-315]	168 [84-289]	0.64
Left ventricular ejection fraction (%), mean (SD)	56.1 (7.6)	54.9 (8.7)	0.26
NT-proBNP (ng/L), median [IQR]	195 [80-452]	183 [97-445]	0.80
Clinical endpoints, n (%) [c]			
MACE [d]	6 (3.2%)	11 (5.9%)	0.22
Cardiovascular mortality	1 (0.5%)	2 (1.1%)	0.57
Non-cardiovascular mortality	1 (0.5%)	0 (0.0%)	0.32
Recurrent myocardial infarction	3 (1.6%)	9 (4.8%)	0.08
STEMI	2 (1.1%)	6 (3.2%)	0.16
NSTEMI	1 (0.5%)	3 (1.6%)	0.32
Recurrent revascularization	4 (2.2%)	5 (2.7%)	0.74
Target-lesion revascularization	3 (1.6%)	3 (1.6%)	0.99
Target-vessel revascularization	0	0	
Non-target-vessel revascularization	1 (0.5%)	2 (1.1%)	0.57
CABG	0	0	
Stent thrombosis	2 (1.1%)	3 (1.6%)	0.66
Stroke	1 (0.5%)	0 (0.0%)	0.32
Hospitalization for heart failure	0 (0.0%)	1 (0.5%)	0.32
Hospitalization for chest pain	6 (3.2%)	3 (1.6%)	0.31
ICD implantation	0	0	

[a] *Analyzed with Beta-Regression*

[b] *Results only shown for CK-MB activity (UMCG), other sites measured CK-MB mass (n=43), these results were consistent with results for CK-MB activity (data not shown).*

[c] *Definitions are available in the Supplementary material*

[d] *Defined as: Cardiovascular death, re-infarction or re-intervention*

Abbreviations: CABG, coronary artery bypass graft surgery; CK-MB, creatine kinase-myocardial band; ICD, Implantable cardioverter-defibrillator; IQR, interquartile range; LV, left ventricle; MACE, major adverse cardiovascular events; NSTEMI, non-ST-segment elevation myocardial infarction; NT-proBNP, N-terminal pro B-type natriuretic peptide; SD, standard deviation; STEMI, ST-segment elevation myocardial infarction; STS, sodium thiosulfate.

Clinical endpoints

At 4 months after randomization no patients were lost to follow-up. Four patients died, 2 in the STS group and 2 in the placebo group. The combined incidence of cardiovascular death, re-infarction and unplanned revascularization at 4 months was 3.2% (6 patients) in the STS group and 5.9% (11 patients) in the placebo group (*P*=0.22). The rates of all other clinical endpoints, including stroke, CVA, hospitalization for heart failure or chest pain are presented in **Table 3**.

Treatment-related adverse effects

Patients in the STS group were more likely to experience nausea and vomiting than those in the placebo group (**Table 4**). Nausea and vomiting continued to occur more frequent in the STS group after the standard use of antiemetics before administration of study medication. A 23 (SD 23) mmHg decline in systolic blood pressure was observed after administration of the first dose of study medication in both treatment arms (*P*=0.55). Blood pressure remained constant after the second dose of study medication in both groups. Other adverse effects were mild and transient. No severe adverse events were observed that were considered related to STS treatment.

Table 4 | Adverse effects

Outcome	STS	Placebo	*P*-value
Serious adverse events, total number, n (%)	18 (9.7%)	18 (9.6%)	0.99
Patients with an adverse event, n (%)	122 (65.6%)	132 (70.6%)	0.30
Adverse events of special interest, n (%)			
1st dose			
New-onset nausea	40 (21.7%)	11 (5.9%)	**<0.001**
New onset vomiting	25 (13.7%)	4 (2.2%)	**<0.001**
Without preventive antiemetics			
New-onset nausea	25 (33%)	8 (12%)	**0.002**
New onset vomiting	13 (17%)	2 (3%)	**0.005**
With preventive antiemetics			
New-onset nausea	15 (13.9%)	3 (2.6%)	**0.002**
New onset vomiting	12 (11.2%)	2 (1.7%)	**0.004**
2nd dose			
New-onset nausea	31 (18.8%)	6 (3.6%)	**<0.001**
New onset vomiting	18 (10.9%)	2 (1.2%)	**<0.001**
Without preventive antiemetics			
New-onset nausea	14 (19%)	2 (3%)	**0.001**
New onset vomiting	7 (9%)	1 (1%)	**0.024**
With preventive use antiemetics			
New-onset nausea	17 (19%)	4 (4%)	**0.002**
New onset vomiting	11 (12%)	1 (1%)	**0.003**

Abbreviation: STS, sodium thiosulfate.

Per-protocol analysis and subgroup analyses

The results of the per-protocol analysis were consistent with the intention to-treat analysis (**Supplementary Table 5**). The results of the primary endpoint were also consistent across pre-specified subgroups (**Figure 2**).

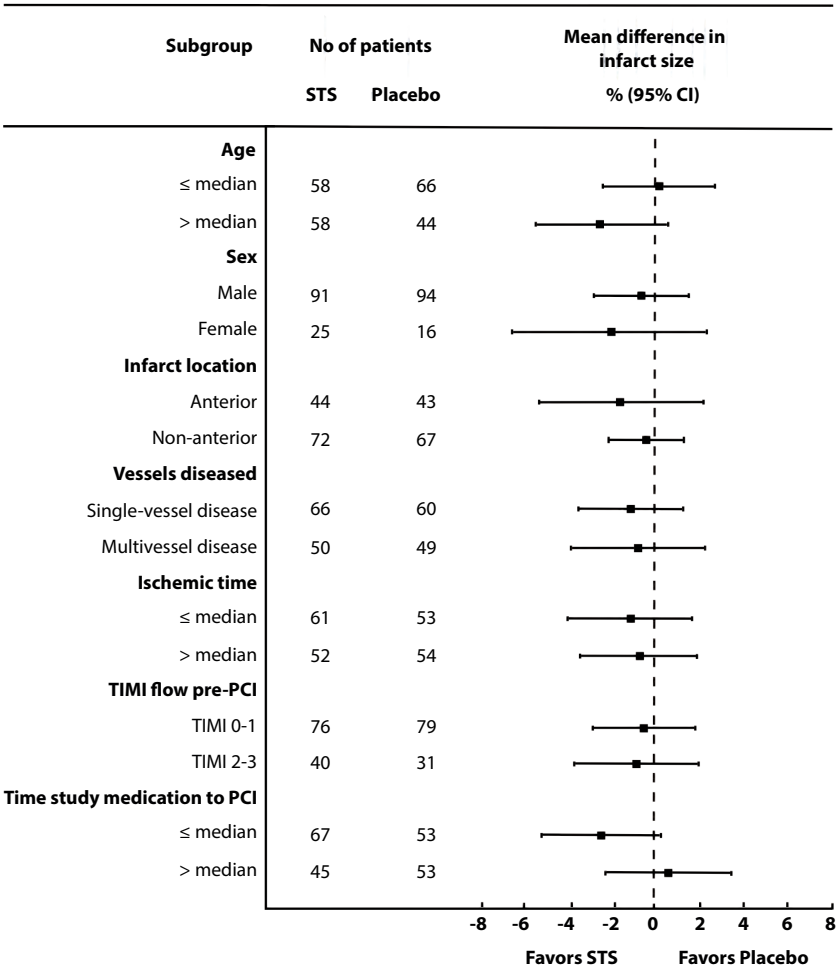
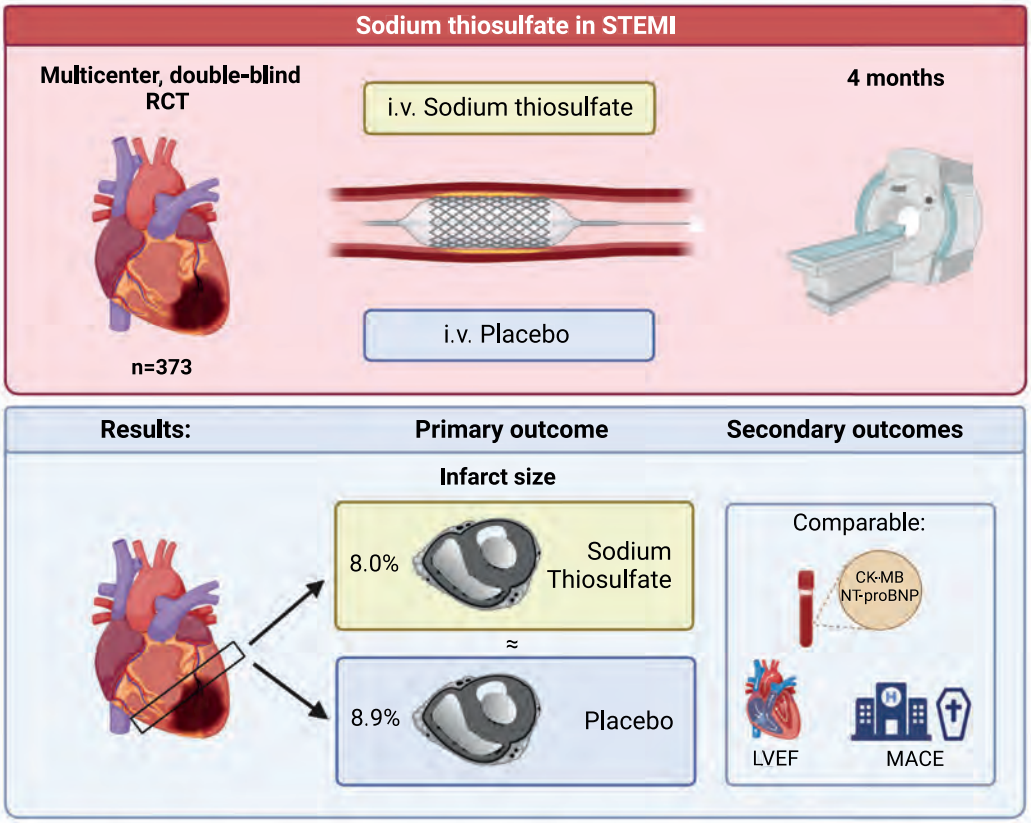


Figure 2 | Effect of sodium thiosulfate on infarct size across prespecified subgroups

Forest plot depicting the estimated treatment effect of sodium thiosulfate on the primary outcome infarct size at 4 months follow-up across prespecified subgroups (age ≤ median (61 years) vs. age > median; male sex vs female sex; anterior myocardial infarction vs. non-anterior myocardial infarction; singe-vessel disease vs. multivessel disease; ischemic time ≤ median (141 minutes) vs. > the median; TIMI flow pre-PCI 0-1 vs. 2-3; and time from start of study medication to PCI ≤ median (16 minutes) vs. > the median). Treatment effects of STS were consistent across all subgroups (P for interaction all >0.05). Abbreviations; CI, confidence interval; PCI, percutaneous coronary intervention; STS, sodium thiosulfate: TIMI, thrombolysis in Myocardial Infarction.

DISCUSSION

Among patients presenting with STEMI, intravenous STS treatment initiated before primary PCI did not reduce myocardial infarct size compared to placebo (**Central illustration**). There was also no effect on left ventricular ejection fraction. Patients in the STS group were more likely to experience nausea and vomiting than those in the placebo group.



Central illustration | Sodium thiosulfate administration in acute myocardial infarction

A total of 373 people with a first STEMI were randomized to receive either sodium thiosulfate 12.5 grams intravenously or matching placebo at admission to the catheterization laboratory and 6 hours thereafter. The differences in infarct size, evaluated by cardiac magnetic resonance imaging at 4 months as well as the enzymatic infarct size during index hospitalization and LVEF, NT-proBNP levels and MACE at 4 months were evaluated. In this population at relatively low risk no significant treatment effects were observed. Abbreviations: LVEF, left-ventricular ejection fraction; MACE, major adverse cardiovascular events; NT-proBNP, N-terminal pro-B-type natriuretic peptide; RCT, randomized controlled trial; STEMI, ST-segment elevation myocardial infarction.

Patients presenting with STEMI are routinely treated with primary PCI to treat myocardial ischemia with reperfusion. Reperfusion to an ischemic area has been associated with cellular injury, which may substantially contribute to the final infarct size.[8,10] The current proof-of-concept study was developed to clinically translate the plethora of pre-clinical studies providing strong mechanistic and functional evidence that STS and H_2S can substantially reduce reperfusion injury.[14,40] For example, substantial infarct sparing effects were observed in both small and large animal models using different H_2S sources.[15,24,40–44] STS has also been shown beneficial in several other clinical settings associated with cellular toxicity (e.g. cyanide intoxication, calciphylaxis, reduction of cisplatin related toxicity). In our study, STS induced the known side-effects such as nausea and vomiting but did not reduce myocardial infarct size.

Several findings may explain the lack of benefit of STS in our patient cohort. First, the final infarct size of our patients was relatively small. This is a consequence of the well-organized STEMI network in the Netherlands with short ischemic time (2-2.5 hours versus 3 hours in other recent studies),[45–47] and pretreatment by the ambulance service with a loading dose of a P2Y12 inhibitor, aspirin and intravenous heparin. Moreover, we also included patients with TIMI 2-3 flow pre-PCI, as the study medication was administered before the start of coronary angiography, bearing in mind that the majority of detrimental effects of I/R injury already occur during the first moments after reperfusion.[8] Potential benefit of STS cannot be excluded in the absence of pretreatment with antiplatelets or in a certain subgroup of patients - for example those presenting with completely closed arteries for extended time, combined with high Killip class, and large area at risk, thus patients with higher probability of additional myocardial salvage.[48] Also, in setting of low availability or unavailability of primary PCI, resulting in delayed reperfusion, STS might reduce myocardial injury. Second, the required cardioprotective concentrations of STS might be higher than could be achieved in this trial. However, the dosage was based on prior efficacy data in humans and was limited by the known side effects.[20,49,50] Furthermore, the amount of STS and H_2S released in the heart during reperfusion remains unknown. Future sub-studies in stored blood samples might provide insight in concentrations and effects on oxidative stress and inflammation. Finally, the duration of treatment was limited to the first hours after reperfusion ($T_{1/2}\approx3$ hours), while reperfusion injury lasts longer.[21,51] Development of oral preparations might enable continued treatment for an extensive period of time, potentially allowing the reported anti-inflammatory, antioxidant and pro-angiogenic properties of H_2S/STS, to modify outcomes.[25,52] Finally, failure to translate preclinical studies into clinical benefit might originate from the absence of co-morbidities and co-medications in animal models.[48]

The incidence of adverse side-effects, mainly nausea and vomiting, was comparable to STS use in other conditions.[19,20] The emetogenic effect of STS did not result in discontinuation of study medication and the additional use of prophylactic antiemetic agents, as was recommended by the data and safety monitoring board (DSMB), appeared to reduce the incidence of nausea and vomiting. The change in blood pressure that we observed in both groups after the first dose of study medication was likely caused by administration of vasodilators, required for the radial PCI procedure, since between-group differences were not observed and no change in blood pressure occurred after the second dose of study medication.

Our trial has several limitations. First, partly due to national covid-19 pandemic restrictions to visit the hospital for non-essential care and fear of patients to acquire a covid-19 infection, the actual percentage of patients who underwent randomization that were available for the primary outcome measure was 59% instead of the anticipated 66%. This led to a reduction of statistical power from the desired 85% to the actual 80%. Post-hoc it seems unlikely that adding ~10 more participants to each arm would have substantially modified our findings. Also the studied number of patients with CMR remains in line with recommendations for the evaluation of cardioprotective strategies.[29] Second, our study was also not powered to detect clinical outcomes such as all-cause mortality or hospitalization for heart failure. However, CMR determined infarct size has been the recommended primary outcome for early assessment of potential cardioprotective therapies.[29] The relevance of our primary outcome is also supported by the reported strong graded response with subsequent mortality and hospitalization for heart failure.[2] We did not take into account area at risk when determining infarct sizes. However, the comparable percentages of proximal culprit lesions (also within each culprit vessel) in both treatment arms (41%) suggest balanced areas at risk. Finally, women were underrepresented and very few patients were non-Caucasian.

In conclusion, we assessed the effect of STS in a proof-of-concept study of patients with STEMI undergoing primary PCI. The administration of STS at time of reperfusion did not lead to a reduction in infarct size. The studied STS dose was not associated with significant adverse events.

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SUPPLEMENTARY MATERIAL

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Supplementary methods II – Definitions clinical endpoints

Mortality
The EAC will adjudicate all subject deaths. Death will be adjudicated for cardiac and all cause death. All deaths are considered cardiac unless an unequivocal non-cardiac cause can be established. Specifically, any unexpected death even in patients with coexisting potentially fatal non-cardiac disease (e.g. cancer, infection) will be classified as cardiac death.

Cardiac death
Cardiac death will be adjudicated for any death due to proximate cardiac cause (eg, MI, low-output failure, fatal arrhythmia), unwitnessed death and death of unknown cause, and all procedure related deaths, including those related to concomitant treatment. A subdivision in cardiac death is made, namely heart failure, sudden cardiac death and other.
Sudden cardiac death is either defined as witnessed, un-witnessed, cardiac arrest without evidence of circulatory collapse, such as hypotension, exacerbation of congestive heart failure, or altered mental status, before the disappearance of the pulse or abrupt collapse occurring within one hour of the onset of the symptoms that resulted in death.
Death due to heart failure will be defined as death due to clinically end-stage heart failure during hospital admission or by exacerbation of congestive heart failure reported by an attending general practitioner.
For all these deaths, no probable non-cardiac cause should be suggested by the history or autopsy;

Non cardiac death
Any death not covered by the above definitions, such as death caused by infection, malignancy, sepsis, pulmonary causes, accident, suicide, or trauma.

Myocardial infarction
The EAC will adjudicate all cases of MI, after the initial index (STEMI) event defined by the documented fall of cardiac markers, and the relationship of the event to the target/culprit vessel and the presence of stent-thrombosis. All infarcts that cannot be clearly attributed to a vessel other than target/culprit vessel will be considered related to the target vessel.
Criteria for Acute Myocardial Infarction (based on the Fourth Universal Definition of Myocardial Infarction Guidelines, ESC 2018):
The term acute myocardial infarction should be used when there is acute myocardial injury with clinical evidence of acute myocardial ischaemia with detection of rise and/or fall of cardiac troponin (cTn) values with at least one value above the 99th percentile of the upper reference limit (URL) , together with at least one of the following:

- Symptoms of myocardial ischaemia;
- ECG changes indicative of new ischaemia (new ST-T changes or new left bundle branch block [LBBB]);
- Development of pathological Q waves in the ECG;

- Imaging evidence of new loss of viable myocardium or new regional wall motion abnormalities in a pattern consistent with an ischaemic aetiology;
- Identification of an intracoronary thrombus by angiography or autopsy (not for types 2 or 3 MIs).
  Post-mortem demonstration of acute athero-thrombosis in the artery supplying the infarcted myocardium meets criteria for type 1 MI.
  Evidence of an imbalance between myocardial oxygen supply and demand unrelated to acute athero-thrombosis meets criteria for type 2 MI.
- Cardiac death in patients with symptoms suggestive of myocardial ischemia and presumed new ischemic ECG changes, but death occurred before cTn were obtained, or before cTn values would be increased meets criteria for (type 3) MI.

Coronary procedure related MI (type 4a and 5)

- Percutaneous coronary interventions (PCI) related MI (≤48 hours after the procedure) is arbitrarily defined by elevation of cTn value > 5 X 99th percentile URL in patients with normal baseline values. Patients with elevated pre-procedural cTn values, in whom the pre-procedural cTn levels are stable (≤20% variation) or falling, must meet a >5 fold increase and manifest a change from the baseline value of >20%. In addition, either (1) new ischaemic ECG changes or (2) development of new pathological Q waves or (3) angiographic findings consistent with a procedural flow-limiting complication or (4) imaging evidence of loss of viable myocardium that is presumed to be new and in a pattern consistent with an ischaemic aetiology.
- Coronary artery bypass grafting (CABG) related MI is arbitrarily defined by elevation of cTn value (> 10 X 99th percentile URL) in patients with normal baseline values Patients with elevated pre/procedural cTn values, in whom the pre-procedural cTn levels are stable (≤20% variation) or falling, must meet a >10 fold increase and manifest a change from the baseline value of >20%. In addition, either (1) development of new pathological Q waves or (2) angiographic findings consistent with a procedural flow-limiting complication such as occlusion of a major epicardial graft or (3) imaging evidence of loss of viable myocardium that is presumed to be new and in a pattern consistent with an ischaemic aetiology.

Isolated development of new pathological Q waves meets the type 4a MI or type 5 MI criteria with either revascularization procedure if cTn values are elevated and rising but less than the pre-specified thresholds for PCI and CABG.
Other types of 4 MI include type 4b MI stent thrombosis and type 4c MI restenosis that both meet type 1 MI criteria.
Post-mortem demonstration of a procedure-related thrombus meets the type 4a MI criteria or type 4b MI criteria if associated with a stent.

STEMI/NSTEMI
Myocardial infarction will be subdived into ST-elevated myocardial infarction and non ST-elevated myocardial infarction.

STEMI
To meet criteria for STEMI, acute chest pain and persistent (>20 min) ST-segment elevation (measured at the J-point) on the electrocardiogram should be present in at least two contiguous leads with ST-segment elevation ≥ 2.5 mm in men < 40 years, ≥2 mm in men ≥ 40 years, or ≥ 1.5 mm in women in leads V2–V3 and/or ≥ 1 mm in the other leads [in the absence of left ventricular (LV) hypertrophy or left bundle branch block (LBBB)].

NSTEMI
Patients with NSTEMI should present with acute chest pain but no persistent ST-segment elevation. ECG changes may include transient ST-segment elevation, persistent or transient ST-segment depression, T-wave inversion, flat T waves or pseudo-normalization of T waves or the ECG may be normal.

Stent thrombosis
The EAC will adjudicate all cases of stent thromboses for confirmation.

Definite stent thrombosis
- Angiographic confirmation of stent thrombosis
The presence of a thrombus that originates in the stent or in the segment 5 mm proximal or distal to the stent and presence of at least 1 of the following criteria within a 48-hour time window:
Acute onset of ischemic symptoms at rest
New ischemic ECG changes that suggest acute ischemia
Typical rise and fall in cardiac biomarkers (refer to definition of spontaneous MI)
Non-occlusive thrombus: Intracoronary thrombus is defined as a (spheric, ovoid, or irregular) non calcified filling defect or lucency surrounded by contrast material (on 3 sides or within a coronary stenosis) seen in multiple projections, or persistence of contrast material within the lumen, or a visible embolization of intraluminal material downstream.
Occlusive thrombus: TIMI 0 or TIMI 1 intra-stent or proximal to a stent up to the most adjacent proximal side branch or main branch (if originates from the side branch).

- Pathological confirmation of stent thrombosis
Evidence of recent thrombus within the stent determined at autopsy or via examination of tissue retrieved following thrombectomy.

Probable stent thrombosis:
Clinical definition of probable stent thrombosis is considered to have occurred after intracoronary stenting in the following cases:
Any unexplained death within the first 30 days, irrespective of the time after the index procedure,
Any MI that is:
related to documented acute ischemia in the territory of the implanted stent without angiographic confirmation of stent thrombosis, and in the absence of any other obvious cause

Possible stent thrombosis:
Clinical definition of possible stent thrombosis is considered to have occurred with any unexplained death from 30 days after intracoronary stenting until end of trial follow-up. We follow the ARC definitions. Stent Thrombosis should be reported as a cumulative value at the different time points and with the different separate timepoints. Time 0 is defined as the time point after the guiding catheter has been removed and the patient left the cathlab.

Timing:
Acute stent thrombosis: 0 to 24 hours after stent implantation
Subacute stent thrombosis: 24 hours to 30 days after stent implantation
Late stent thrombosis: 30 days to 1 year after stent implantation
Very late stent thrombosis: 1 year after stent implantation

Revascularisation
Any repeat revascularization procedure (PCI or CABG) will be considered as an event, except for scheduled revascularization based on the index CAG to diagnose and treat coronary artery lesions identified during the index procedure and heart team discussion. In case a scheduled revascularization based on the index CAG to treat coronary artery lesions identified during the index procedure will be performed earlier than originally scheduled due to an urgent medical reason (for example ongoing chest pain) it will be adjudicated as recurrent revascularization. In case the scheduled revascularization is performed earlier without urgent medical reason, it will not be adjudicated as a recurrent revascularization.

Target Lesion Revascularization (TLR)
TLR is defined as a repeat percutaneous intervention of the target/culprit lesion of the index CAG. The target lesion is defined as the treated segment from 5 mm proximal to the stent and to 5 mm distal to the stent.

Target Vessel Revascularisation (TVR)
TVR is defined as a repeat percutaneous intervention in the same coronary artery as the index procedure (which includes upstream and downstream branches), but not in the same coronary segment (defined as the treated segment from 5mm proximal to the stent and 5 mm distal to the stent).

Non Target Vessel Revascularisation (NTVR)
Any repeat percutaneous intervention not covered by the above definitions for target lesion and target vessel revascularization.
Coronary artery bypass grafting
New therapeutic coronary bypass grafting after the index procedure.

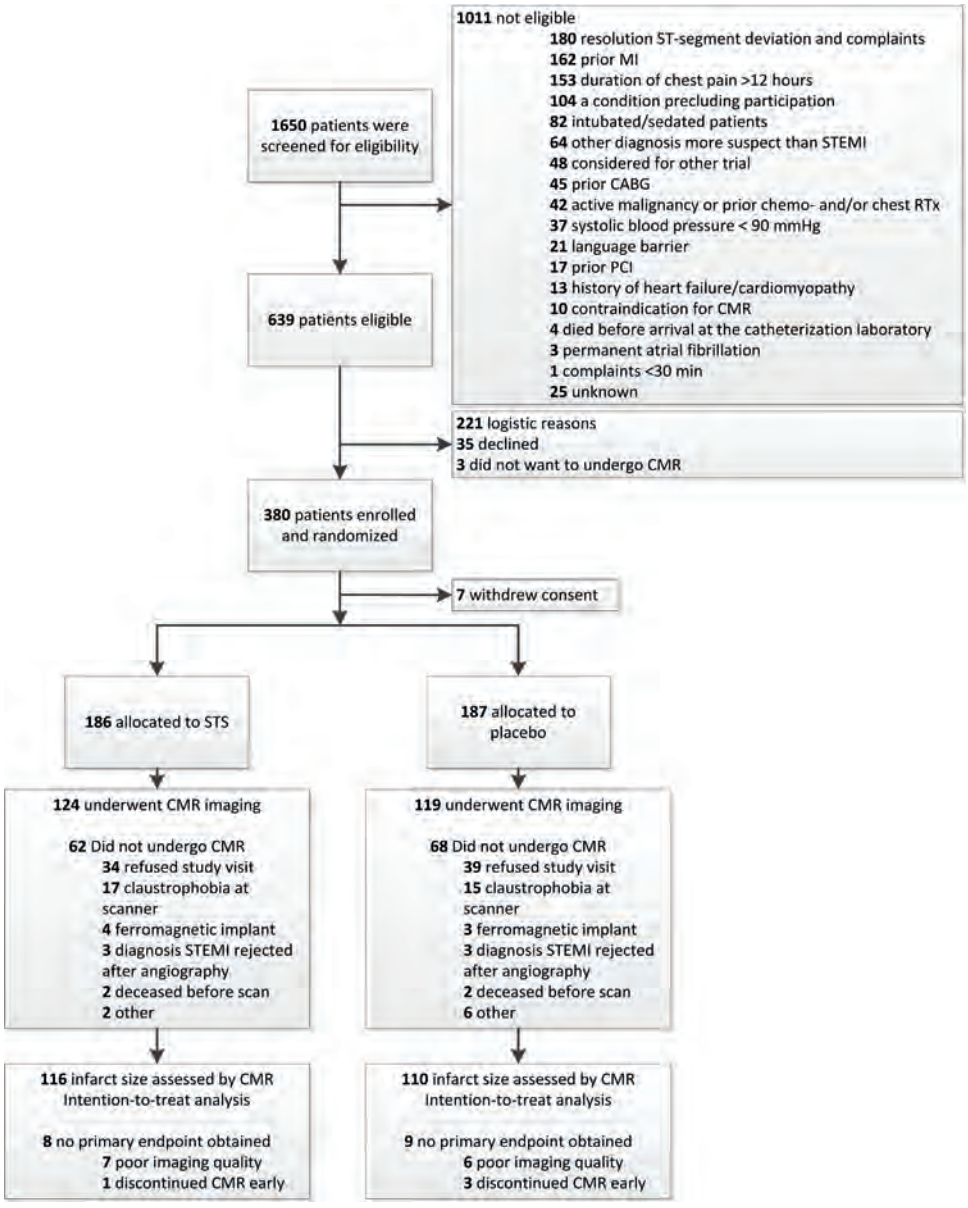
Cerebrovascular accident
Acute neurological event of at least 24 hours of duration, with focal signs and symptoms and without evidence supporting any alternative explanation. Diagnosis of stroke requires confirmation by CT or MRI or pathological confirmation. Stroke is further classified as ischemic, hemorrhagic or type uncertain. Ischemic stroke occurs as a result of an obstruction within a blood vessel supplying blood to the brain. Hemorrhagic stroke includes intraparenchymal, subarachnoid hemorrhage and subdural hematomas.

Hospitalization
Hospitalization is defined as an non-elective admission to the hospital after discharge from the index event with overnight stay (different dates for admission and discharge).

Hospitalization for heart failure
Signs and symptoms consistent with heart failure, confirmed by clinical findings and laboratory parameters, and;
No alternative clinical explanation for the signs and symptoms.

Hospitalization for chest pain
Recurrent signs and symptoms suggestive of ischemia, and;
Absence of new or recurrent ST-segment elevations or depression suggestive of MI, and;
No rise of biochemical markers of myocardial necrosis (including troponin, CK-Mb, CK) to above the upper limit of normal (or if markers already elevated, greater than 50% of the lowest recovery enzyme level from the index infarction), and;
No alternative clinical explanation for the signs and symptoms.

ICD-implantation
Implantation of an Internal Cardiac Defibrillation after the index event.



Supplementary Figure 1 | Trial flowchart

Abbreviations: CABG, coronary artery bypass graft; CMR, cardiac magnetic resonance imaging; MI, myocardial infarction; PCI percutaneous coronary intervention; STEMI, ST-elevation myocardial infaction; STS, sodium thiosulfate; RTx, radiotherapy.

Supplementary Table 1 | Medication at discharge

Medication at discharge	STS n=185 [a]	Placebo n=186 [a]
Aspirin	180 (97.3%)	178 (95.7%)
Ticagrelor	151 (81.6%)	152 (81.7%)
Clopidogrel	29 (15.7%)	25 (13.4%)
Prasugrel	1 (0.5%)	0
Beta-blocker	168 (90.8%)	168 (90.3%)
ACE-inhibitor or angiotensin receptor blocker	153 (82.7%)	158 (84.9%)
Statin	177 (95.7%)	179 (96.2%)
Calcium antagonist	16 (8.6%)	24 (12.9%)
Diuretics	25 (13.5%)	21 (11.3%)
Oral anticoagulants		
Direct oral anticoagulants	8 (4.3%)	6 (3.2%)
Vitamin K antagonist	4 (2.2%)	5 (2.7%)

[a] Two patients died during hospitalization (1 in the STS group, 1 in the placebo group)

Abbreviations: ACE, angiotensin converting enzyme; STS, sodium thiosulfate.

Supplementary Table 2 | Baseline characteristics of the CMR population

Characteristic	STS n =116	Placebo n =110
Demography		
Age at randomization (years), mean (SD)	61.9 (10.7)	59.1 (11.3)
Male sex, n (%)	91 (78.4%)	94 (85.5%)
Body Mass Index (kg/m^2), mean (SD)	27.1 (3.9)	27.2 (4.2)
Caucasian ethnicity, n (%)	111 (95.7%)	107 (97.3%)
Prior conditions, n (%)		
Hypertension	53 (45.7%)	43 (39.1%)
Dyslipidemia	35 (30.2%)	37 (33.6%)
Current smokers	38 (32.8%)	39 (35.5%)
Positive family history	48 (41.7%)	51 (46.4%)
Diabetes Mellitus	14 (12.1%)	10 (9.1%)
Previous MI	1 (0.9%)	0
Previous PCI	2 (1.7%)	1 (0.9%)
Clinical characteristics		
Systolic blood pressure, mmHg, mean (SD)	139 (26)	143 (23)
Diastolic blood pressure, mmHg, mean (SD)	83 (17)	87 (15)
Heart rate, bpm, mean (SD)	73 (17)	75 (16)
Killip class I, n (%)	106 (97.2%)	107 (97.3%)
Laboratory parameters, median [IQR]		
Hemoglobin (mmol/L)	8.7 [8.1-9.2]	8.9 [8.3-9.3]
Creatinine (µmol/L)	74 [65-84]	78 [64-86]
CK (U/L)	135 [91-208]	144 [90-242]
CK-MB activity (U/L)	16 [13-20]	16 [13-23]
NT-proBNP (ng/L)	78 [39-187]	72 [33-192]
Glucose (mmol/L)	5.7 [5.5-6.0]	5.5 [5.4-5.9]

Baseline characteristics stratified by treatment allocation.

Abbreviations: bpm, beats per minute; CK, creatine kinase; CK-MB, creatine kinase-myocardial band; CMR, cardiac magnetic resonance imaging; IQR, interquartile range; MI, myocardial infarction; NT-proBNP, N-terminal pro-B-type natriuretic peptide; PCI, percutaneous coronary intervention; SD, standard deviation; STS, sodium thiosulfate.

Supplementary Table 3 | Procedural characteristics of the CMR population

Characteristic	STS n =116	Placebo n =110
Time from symptom onset to start study medication, (min), median [IQR]	118 [77-175]	126 [90-220]
Time from symptom onset to wire passage, (min), median [IQR]	134 [97-178]	142 [104-244]
Single vessel disease, n (%)	66 (56.9%)	60 (54.5%)
Culprit territory, n (%)		
Left anterior descending	48 (41.4%)	47 (42.7%)
Circumflex or marginal	23 (19.8%)	18 (16.4%)
Right coronary artery	44 (37.9%)	43 (39.1%)
Left main	0	1 (0.9%)
No clear culprit	1 (0.9%)	1 (0.9%)
Medication from first medical care to PCI, n (%)		
Aspirin	116 (100%)	109 (99.1%)
Loading dose of P2Y12 inhibitor	116 (100%)	110 (100%)
Heparin	116 (100%)	110 (100%)
Glycoprotein IIb/IIIa inhibitor	20 (17.2%)	17 (15.5%)
TIMI flow grade pre-PCI, n (%)		
0	69 (59.5%)	73 (66.4%)
1	7 (6.0%)	6 (5.5%)
2	19 (16.4%)	10 (9.1%)
3	21 (18.1%)	21 (19.1%)
Proximal lesion, n (%)	44 (37.9%)	43 (39.1%)
Initial intervention of culprit lesion, n (%)		
PCI	114 (98.3%)	108 (98.2%)
CABG	1 (0.9%)	0 (0.0%)
Conservative	1 (0.9%)	2 (1.8%)
No reflow observed on angiography, n (%)	3 (2.6%)	5 (4.6%)
Distal embolization after PCI, n (%)	10 (8.8%)	5 (4.6%)
TIMI flow grade post-PCI, n (%)		
0	4 (3.5%)	3 (2.8%)
1	3 (2.6%)	0 (0.0%)
2	2 (1.8%)	6 (5.6%)
3	105 (92.1%)	99 (91.7%)

Abbreviations: CABG, coronary artery bypass graft; CMR, cardiac magnetic resonance imaging; PCI, percutaneous coronary intervention; STS, sodium thiosulfate; TIMI, Thrombolysis in Myocardial Infarction.

Supplementary Table 4 | Medication at discharge in the CMR population

Medication at discharge	STS n=116	Placebo n=110
Aspirin	116 (100%)	109 (99.1%)
Ticagrelor	96 (82.8%)	100 (90.9%)
Clopidogrel	18 (15.5%)	9 (8.2%)
Beta-blocker	107 (92.2%)	99 (90.0%)
ACE-inhibitor or angiotensin receptor blocker	98 (84.5%)	95 (86.4%)
Statin	114 (98.3%)	108 (98.2%)
Calcium antagonist	7 (6.0%)	9 (8.2%)
Diuretics	12 (10.3%)	8 (7.3%)
Oral anticoagulants		
Direct oral anticoagulants	4 (3.4%)	2 (1.8%)
Vitamin K antagonist	1 (0.9%)	2 (1.8%)

Abbreviations: ACE, angiotensin converting enzyme; CMR, cardiac magnetic resonance imaging; STS, sodium thiosulfate.

Supplementary Table 5 | Per-protocol analysis

Outcome	STS	Placebo	*P*-value
Infarct size, % of LV mass, mean (SD)	7.5 (6.5)	8.8 (7.5)	0.22 [a]

[a] Analyzed with Beta-regression. The Marginal average difference in infarct size was -1.1% (95% confidence interval -3.1% to 0.7%) for the STS group, compared with the placebo group.
Abbreviations: LV, left ventricle; SD, standard deviation; STS, sodium thiosulfate.