Randomized Phase 2 Trial of Intracoronary Nitrite During Acute Myocardial Infarction

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Rationale: **Preclinical evidence demonstrates that inorganic nitrite, after its in situ conversion to nitric oxide, attenuates consequent myocardial reperfusion injury.**

Objective: **We investigated whether intracoronary injection of nitrite during primary percutaneous coronary intervention might improve infarct size in ST-elevated myocardial infarction.**

Methods and Results: **Patients undergoing primary percutaneous coronary intervention (n=80) were randomized to receive intracoronary (10 mL) sodium nitrite (1.8 μmol) or NaCl (placebo) before balloon inflation. The primary end point was infarct size assessed by measuring creatine kinase release. Secondary outcomes included infarct size assessed by troponin T release and by cardiac MRI on day 2. Baseline characteristics were similar between the groups. No evidence of differences in creatine kinase release (*P*=0.92), troponin T (*P*=0.85), or cardiac MRI–assessed infarct size (*P*=0.254) were evident. In contrast, there was an improvement in myocardial salvage index (*P*=0.05) and reduction in major adverse cardiac event at 1 year (2.6% versus 15.8%; *P*=0.04) in the nitrite group. In a 66-patient subgroup with thrombolysis in myocardial infarction ≤1 flow, there was reduced serum creatine kinase (*P*=0.030) and a 19% reduction in cardiac MRI–determined infarct size (*P*=0.034) with nitrite. No adverse effects of nitrite were detected.**

Conclusions: **In this phase II study, intracoronary nitrite infusion did not alter infarct size, although a trend to improved myocardial salvage index and a significant reduction in major adverse cardiac event was evident. In a subgroup of patients with thrombolysis in myocardial infarction flow ≤1, nitrite reduced infarct size and major adverse cardiac event and improved myocardial salvage index, indicating that a phase III clinical trial assessing intracoronary nitrite administration as an adjunct to percutaneous coronary intervention in ST-elevated myocardial infarction patients is warranted.**

Clinical Trial Registration: **URL: http://www.clinicaltrials.gov. Unique identifier: NCT01584453.**

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Key Words: acute myocardial infarction ■ nitric oxide ■ percutaneous coronary intervention

ST-segment elevation myocardial infarction (STEMI) is thought to account for ≈25% to 47% of all acute myocardial infarctions (AMI).[1,2] Presently, timely and effective reperfusion with primary percutaneous coronary intervention (PCI) is the treatment of choice for reducing infarct size, preserving left ventricular ejection fraction, and preventing the onset of heart failure.[3,4] Myocardial reperfusion injury may account for ≤50% of final myocardial infarct size and is a major determinant of prognosis,[5] and this underlies interest in targeting reperfusing injury using adjunctive pharmacotherapy.

Although several therapeutic interventions have been tested in this regard and failed, in more recent years an improved understanding of the pathophysiological mechanisms underlying ischemia/reperfusion injury has resulted in identification of some promising mechanical (ischemic postconditioning,[6] remote ischemic preconditioning)[7] and pharmacological (cyclosporine,[8] exenatide)[9] strategies.[10] More recently, an additional possibility has emerged in the form of inorganic nitrite. The activity of nitrite resides in its conversion to nitric oxide (NO) under the optimal conditions of low PO_2 and pH,[11] conditions

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Nonstandard Abbreviations and Acronyms	
AAR	area at risk
AUC	area under the curve
CMR	cardiac MRI
MACE	major adverse cardiac event
MVO	microvascular obstruction
NO	nitric oxide
PCI	percutaneous coronary intervention
STEMI	ST-segment elevation myocardial infarction
TIMI	thrombolysis In Myocardial Infarction

that prevail during ischemic episodes. NO exerts several beneficial effects, including anti-inflammatory and antiplatelet actions, and prevents the opening of the mitochondrial permeability transition pore, which is a critical and final common step in reperfusion.[12,13]

We first demonstrated the cardioprotective effects of intracoronary nitrite in isolated rat hearts,[14] an observation likewise demonstrated by others with intraventricular and intracoronary nitrite administration both in vitro and in vivo.[15–18] In all of these studies, the beneficial effects were attributed to NO and were more often associated with local rather than systemic application of high local concentrations of nitrite (3–12 μmol/L), before reperfusion. Together, these observations provide the rationale for the investigation of the therapeutic potential of nitrite in the treatment of acute STEMI, where nitrite is delivered locally before balloon inflation at the time of primary PCI.

Methods

Study Design and Participants

This study was a double-blind, randomized, single-center, placebo-controlled trial to determine whether intracoronary injection of sodium nitrite reduces infarct size in patients with acute STEMI undergoing primary PCI. The trial was approved by an independent ethics committee, the Medicines and Healthcare Products Regulatory Agency, registered in approved registries (NCT01584453, EudraCT nr. 2011-000721-77) and performed in accordance with the Declaration of Helsinki (1996) and the principles of the International Conference on Harmonization–Good Clinical Practice guidelines. Full details of the trial protocol have been published.[19] All appropriate subjects gave written informed consent before being included in the study (see Online Data Supplement for further details). Consent in the emergency situation is challenging, and thus, patients who were unconscious, critically unstable (cardiogenic shock), or deemed unable to consent (pain, distress, language) were excluded. Consent was a 2-stage process where initially a study summary sheet consisting of a 1-page sheet with diagrams, explaining the procedure and events, was given to the patient before randomization and full written consent taken at this time. A more detailed patient information sheet was given after the procedure for reading. A second stage of consent was required for agreement to subsequent cardiac MRI (CMR) analyses (see below).

All consecutive patients presenting to Barts Health Heart Attack Center, based at The London Chest Hospital, suspected of an acute STEMI, and candidates for primary PCI were considered eligible for participation. Inclusion criteria were symptoms of chest pain suggestive of myocardial ischemia, time from onset of symptoms of ≤6 hours, aged between 18 and 80 years of age, and an ECG showing ST-segment elevation of 0.1 mV in ≥2 limb leads or 0.2 mV in ≥2 contiguous precordial leads or presumed new left bundle branch block.

Patients with cardiac arrest, cardiogenic shock, previous AMI, or CABG were not included in the study. Patients with known congenital methemoglobinemia, left ventricular systolic dysfunction caused by preexisting heart failure, chronic renal failure (ie, with an estimated glomerular filtration rate <30 mL/min), and women who were pregnant were not included. Finally, patients on preexisting treatment with organic nitrate therapy (Nicorandil, isosorbide mononitrate), or had active malignancy, a life-threatening condition, or had participated in any investigational drug or device study within the past 30 days were excluded.

Randomization and Intervention

After coronary angiography, patients were randomized (1:1) to a high-dose bolus injection of intracoronary sodium nitrite (1.8 μmol in 10 mL of 0.9% NaCl) or placebo (10 mL of 0.9% NaCl) administered before balloon inflation. After crossing the obstruction of the infarct-related coronary artery with a guidewire, an over-the-wire balloon (Emerge, Boston Scientific, Natick, MA, USA) was positioned beyond the obstruction. The guidewire was removed and the study drug solution injected by hand through the central lumen of the balloon catheter into the distal vascular bed over 30 seconds, irrespective of thrombolysis in myocardial infarction (TIMI) flow beyond the occlusion point. The guidewire was then reinserted through the balloon catheter and advanced to a distal position. The procedure was then continued as per operator preference with no restriction placed on vascular access route, type of stent or method of stenting (predilatation or direct).

The dose of nitrite administered was chosen because studies in the forearm of healthy volunteers demonstrate bioactivity of local concentrations of 2.5 to 10 μmol/L after bolus administration[20–22]: a range broadly associated with cardioprotection in reperfusion injury achieved through bolus dose administration in preclinical studies.[15,18] Manufacture of the interventions, blinding, coding, and randomization were conducted by the Pharmacy Manufacturing Unit at Ipswich Hospital before transfer to the London Chest Hospital Pharmacy. The randomization list was computer-generated based on blocks of 10 and kept in a sealed opaque envelope in the hospital pharmacy. No stratification factors were used. A total of 80 indistinguishable vials of sodium nitrite and placebo were provided. All study personnel were blind to treatment allocation until the study, and all analyses had been completed. All patients underwent standard UK and Barts Health Trust care protocols before and post primary PCI (Table 1).

End Points

Primary End Point

The primary end point was infarct size assessed by measurement of area under the curve (AUC) for creatine kinase (CK) in line with previous studies.[8]

Secondary End Points

The principal secondary end point was infarct size assessed by troponin T AUC and the area of delayed hyperenhancement evident by CMR, assessed on day 2 and 6 months after infarction.

Assessment of Infarct Size

Blood samples were obtained at admission and repeatedly over the next 2 days after treatment as per Barts Health Trust protocols. The AUC (expressed in arbitrary units) for CK and troponin T was measured in each patient by computerized planimetry (GraphPad prism v5.0, California).

Infarct size was also assessed using CMR. A CMR scan was offered as a substudy with separate consent to participants and was conducted according to standard protocols (see Online Data Supplement for detail). CMR-related measures in addition to the above-mentioned secondary end points were area at risk (AAR), myocardial salvage index, microvascular obstruction (MVO), left ventricular volumes, and ejection fraction. The latter 2 are conventional measures of cardiac function; the rest of the measures provide some insight into the potential mechanisms involved in any beneficial effects that might be seen.

Coronary Angiography and Subgroup

Coronary angiograms obtained before and after primary PCI were used to make an assessment of TIMI flow grade and AAR using

Table 1. Baseline Characteristics of the Study Population

	Nitrite (n=40)	Placebo (n=40)
Age, y (mean±SD)	56.35±11.16	57.60±13.20
Sex (M/F)	36/4	31/9
Diabetes mellitus	3 (7.5%)	3 (7.5%)
Body-mass index, kg/m^2 (mean±SD)*	28.97±5.14	28.58±5.17
Hypertension	20 (50.0%)	14 (35.0%)
Hypercholesterolemia	16 (40.0%)	12 (30.0%)
Heart rate, bpm (mean±SD)	72.68±18.62	77.35±21.31
Systolic BP, mm Hg (mean±SD)	124.48±29.92	136.13±26.99
Ischemia time, min (mean±SD)†	207.05±76.34	171.63±67.72
Door to balloon time, min (mean±SD)	46.45±13.76	42.35±11.94
Culprit vessel		
Left anterior descending	9 (22.5%)	12(30%)
Circumflex	5 (12.5%)	5 (12.5%)
Right coronary	26 (65.0%)	23 (57.5%)
TIMI flow before PCI		
0	30 (75.0%)	31 (77.5%)
1	6 (15.0%)	3 (7.5%)
2	2 (5.0%)	5 (12.5%)
3	2 (5.0%)	1 (2.5%)
0/1	36 (90.0%)	34 (85.0%)
Syntax score (mean±SD)	13.41±5.50	13.58±6.20
DES use	33 (82.5%)	30 (78.9%)
Treatment before PCI		
Morphine	26 (43.3%)	34 (56.7%)
Treatment at time of PCI		
Heparin	40 (100%)	40 (100%)
Aspirin	40 (100%)	40 (100%)
Clopidogrel/prasugrel (No/No)	35/5	37/3
Glycoprotein IIb/IIIa inhibitor	40 (100%)	40 (100%)

Values shown as number (%) unless otherwise stated.

BP indicates blood pressure; DES, drug-eluting stent; PCI, percutaneous coronary intervention; and TIMI, thrombolysis in myocardial infarction.

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

†Ischemia time determined from symptom to balloon times for each patient.

standard (BARI and APPROACH) validated approaches (see Online Data Supplement for detail). The TIMI flow assessments were then used to make an assessment of inclusion for a single substudy analysis of the effect of nitrite in only patients with TIMI flow ≤1 at revascularization. This subgroup was specifically assessed because evidence demonstrates that cardioprotective strategies are most effective in such cohorts of STEMI patients[7] and because the biochemistry of nitrite reduction indicates that activity of the anion is greatest in hypoxic environments.

Safety and Tolerability

Six months and 1 year after AMI, major adverse cardiac events (MACE) (defined as death, myocardial infarction, recurrent revascularization, stroke, and heart failure) were recorded. MACE was assessed at clinic follow-up at 6 months and by telephone follow-up by trained research co-ordinators at 1 year. All events were verified with source documentation. Further safety measures included assessment of the acute safety and tolerability of intracoronary nitrite (hemodyamics and level of methemoglobin) and the incidence of major adverse events occurring within the first 48 hours after reperfusion, including death, heart failure, AMI, stroke, recurrent ischemia, need for repeat revascularization, renal/hepatic insufficiency, vascular complications, and bleeding (see Online Data Supplement for details).

Measurement of Platelet Reactivity and Assessment of Nitrite

Because nitrite has been shown to have important antiplatelet effects, additional hypothesis-generating biochemical and functional assessments of platelet function were made.[23,24] These included assessments of platelet aggregation and P-selectin expression at baseline, 30 minutes post delivery of nitrite/placebo, 4, 24 hours, and 6 months after infarction (see Online Data Supplement for details). In addition, to confirm successful administration of nitrite, circulating plasma nitrite/nitrate levels (collectively termed NOx) were measured at baseline and 30 minutes post delivery of the study drug (see Online Data Supplement for details). Analysis of local coronary concentration after intervention administration was not possible because of the nature of the PCI procedure.

Statistical Analysis

Primary End Point

We hypothesized that nitrite would reduce the CK AUC by 30%, as per previous cardioprotective strategies namely cyclosporine[8] and postconditioning.[6] We chose to assess CK rather than CK-MB because this matches previous studies[6,8] and because CK measurement is part of the routine clinical assessments made in the United Kingdom after PCI. Moreover, although CK-MB might be considered more specific for cardiac injury, recent evidence suggests that CK AUC is comparable to CK-MB, Trop T, or Trop I for the assessment of infarct size.[25] For a statistical power of 80% and a probability of a type I error of 0.05 using a 2-sided test, we calculated that the sample size should be 70 subjects (35 per group). Because 4% to 8% of patients will die by the time of the end point at 6 months and 10% will either not tolerate or fail to attend the CMR at 6 months, an additional 10 patients were recruited to account for these eventualities, giving a total of 80 patients.

Secondary End Point

Based on the assumption of a predicted relative decrease of CMR-determined infarct size of 20% (as per previous studies),[8,26] calculations determined that 31 patients were needed in each patient group (statistical power of 80% and a probability of a type I error of 0.05 using a 2-sided test assuming mean infarct of 25 G and a SD of 7 G).

Analysis was based on the intention-to-treat principle. Baseline demographic and clinical variables were summarized for each arm of the study. Descriptive summaries of the distributions of continuous baseline variables are presented in terms of percentiles (eg, median, 25th, and 75th percentile), whereas discrete variables are summarized in terms of frequencies and percentages.

Comparisons are between the sodium nitrite–treated and placebo control–treated group for the primary and secondary outcomes. The statistical comparison between the treatment groups for the primary end point of CK AUC was performed using the Wilcoxon rank-sum test for nonparametric data because previous studies clearly demonstrate a non-normal distribution for this biomarker.[8]

For all other hypothesis-generating outcome measures, statistical comparisons between the groups were performed using unpaired Student *t* test for data with a normal distribution or Wilcoxon rank-sum tests for data with a non-normal distribution. For comparisons of platelet reactivity between treatments data are expressed as mean±standard error and analysis performed using 2-way repeated measures ANOVA.

To explore mechanisms, associations between indices were measured using Pearson's correlation coefficient with 95% confidence intervals to ascertain whether the CMR measures of infarct size were associated with biochemical measures of infarct size, to determine

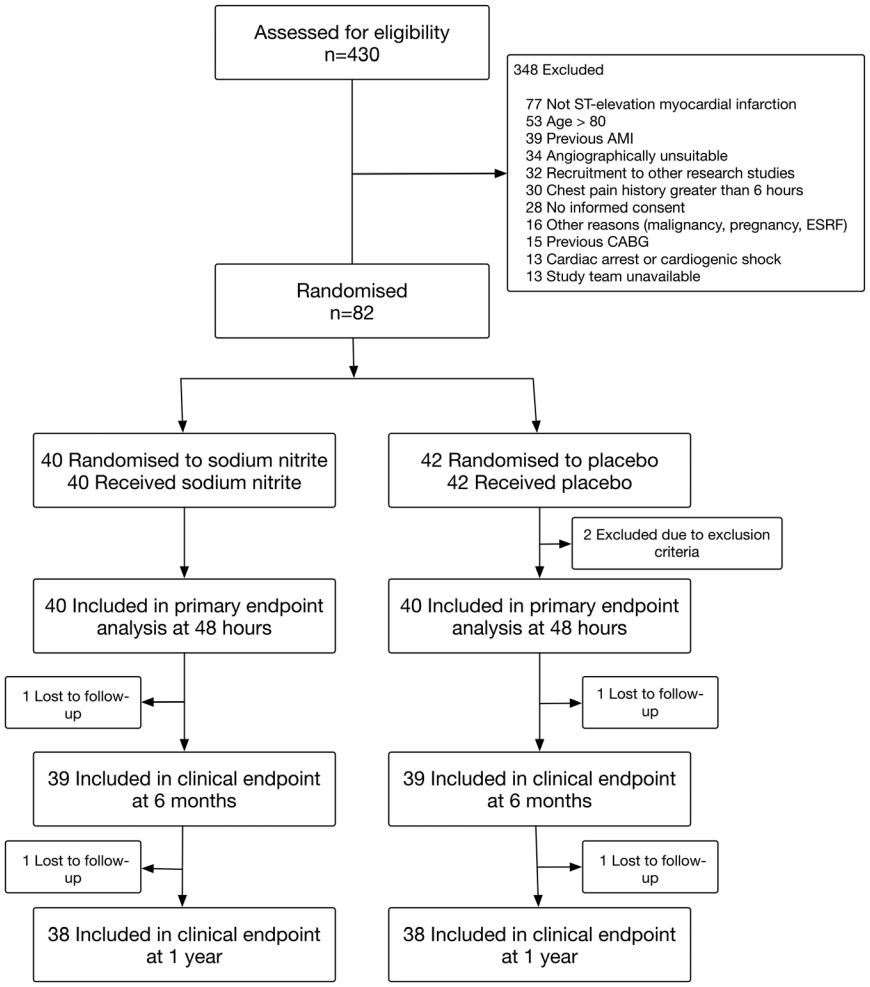

Figure 1. Trial profile. AMI indicates acute myocardial infraction; CABG, coronary artery bypass graft; and ESRF, end-stage renal failure.

whether angiographic measures of AAR were associated with the CMR assessment of AAR, and to determine whether infarct size was associated with platelet reactivity. Statistical significance was established at $P<0.05$ (2-tailed) for all tests and performed using SPSS version 19 (SPSS Inc, Chicago, Ill).

Results

Characteristics of Study Population

Between April 2012 and December 2012, 430 patients were hospitalized for management of AMI at The Barts Health Heart Attack Center. Of these patients, 353 underwent PCI. Among these 353 patients, 13 were not evaluated for enrollment because study personnel were not available. Another 251 were evaluated and excluded as depicted in Figure 1. This left 89 suitable patients, of which 9 declined. Data are thus presented for 80 patients (40 in the control group and 40 in the nitrite group, Figure 1).

All baseline and procedural characteristics were similar between the treatment groups, except ischemia time (Tables 1 and 2). The mean age of the trial participants was 57 years, with 84% male. Twenty-five percent of the cohort had anterior infarcts with similar numbers in both treatment groups. Stenting of the culprit lesion was performed in 97.5% of all patients. In 5 patients, TIMI 3 flow was not achieved after PCI (3 in the nitrite group and 2 in placebo).

Infarct Size

There was no evidence of a difference in the CK AUC between the nitrite and control groups, with a median of 56 398 arbitrary units (IQR, 31 185–83 531) in the nitrite group versus 48 195 (IQR, 27 726–82 841) in the control group ($P=0.92$). The median AUC for troponin T release was 140 782 arbitrary units (IQR, 84 949–218 133) in the nitrite group and 136 412 arbitrary units (IQR, 70 045–239 483) in the control group. This difference was not statistically different ($P=0.85$).

Of the 80 patients recruited, 12 declined consent for the CMR protocols. In the remaining 68 patients, no evidence of a difference in left ventricular volumes, mass, or ejection fraction between the nitrite- and placebo-treated groups were evident (Table 3). However, myocardial salvage index was improved in the nitrite group compared with placebo, although this difference fell on the borders of conventional statistical significance ($P=0.05$; Figure 2A). There was also a trend to smaller infarct size, incidence, and MVO in the nitrite compared with placebo-treated group. CMR-assessed infarct size was positively associated with cardiac biomarkers (CK, $r=0.770$, $P<0.01$; troponin T, $r=0.787$, $P<0.01$; Online Figure I). The CMR-assessed AAR was associated with both angiographic risk scores (APPROACH, $r=0.678$, $P<0.01$; BARI, $r=0.541$, $P<0.01$; Online Figure II).

Table 2. Delivery of IMP, Procedural and Clinical Outcomes

	Nitrite (n=40)	Placebo (n=40)	*P* Value
IMP Delivery			
Systolic BP drop, median (IQR)	8.8 (0.5–30.1)	11.0 (2.1–21.4)	0.90
Systolic BP drop >10%	18 (45%)	23 (57.5%)	0.37
MetHb, median (IQR)	0.1 (0–0.13)	0 (0–0.2)	0.66
Angiographic AAR			
APPROACH, mean (95% CI)	30.89 (28.30–33.47)	26.63 (23.28–29.99)	0.05
BARI, mean (95% CI)	27.41 (24.02–30.80)	25.17 (22.20–28.13)	0.32
Contrast	261.20±15.61	236.30±12.05	0.21
ST segment resolution (>70%)	5 (88.5%)	5 (88.5%)	0.99
Manual thrombectomy	33 (82.5%)	31 (77.5%)	0.78
Procedural success	37 (92.5%)	38 (95.0%)	0.62
Clinical events			
48 hours	3 (7.5)	7 (17.5)	0.31
Death	0 (0)	0 (0)	
Recurrent Ischemia	0 (0)	1 (2.5)	
Heart failure	2 (5)	3 (7.5)	
CIN	1 (2.5)	3 (7.5)	
6 months	N=40	n=40	
MACE	0 (0)	4 (10.0)	0.04
Death	0 (0)	0 (0)	
Repeat revascularization	0 (0)	2 (5.0)	
Recurrent myocardial infarction	0 (0)	1 (2.5)	
Hospitilization for heart failure	0 (0)	1 (2.5)	
1 year	N=38	N=38	
MACE	1 (2.6%)	6 (15.8%)	0.04
Death	0 (0)	0 (0)	
Repeat revascularization	1 (2.6%)	3 (7.9)	
Recurrent myocardial infarction	0 (0)	1 (2.6)	
Hospitilization for heart failure	0 (0)	2 (5.3)	
Medication at 1 year			
Beta-blocker	33 (91.7%)	32 (88.9%)	0.69
ACE-i	33 (91.7%)	31 (86.1%)	0.45
ARB	3 (8.3%)	5 (13.9%)	0.45
Statin	36 (100%)	36 (100%)	1.00
Aspirin	36 (100%)	35 (97.2%)	0.31
ADP antagonist	30 (83.3%)	29 (80.6%)	0.76

Values shown as number (%) unless otherwise stated.

AAR indicates area at risk; ACE-i, angiotensin-converting enzyme inhibitor; ADP, adenosine diphosphate; APPROACH, Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease; ARB, angiotensin receptor blocker; BARI, Bypass Angioplasty Revascularization Investigation; BP, blood pressure; CI, confidence interval; CIN, contrast-induced nephropathy; IMP, investigational medicinal product; IQR, interquartile range; and MACE, major adverse cardiac events.

Coronary Angiography and Subgroup Analysis

Because nitrite bioactivity is thought to occur to a greater extent under ischemic conditions, we assessed the effect of nitrite on infarct size according to whether the culprit vessel was occluded or not at the time of drug administration. Angiographic analysis indicated that 66 of the 80 patients had TIMI flow ≤1 preprocedure and successful drug delivery (ie, unsuccessful procedures excluded). In this subgroup, ischemia time was the same between the 2 groups as were all other baseline characteristics (Online Table I). Importantly, in this subgroup, there was a significant reduction in myocardial infarct size assessed by CK AUC between the nitrite and control groups, with a median of 44 608 arbitrary units (IQR, 27 535–64 848) in the nitrite group versus 55 666 (IQR, 41 591–93 659) in the control group (*P*=0.030). This represents a 19% reduction in infarct size (Figure 3A). The median AUC for troponin T release was 131 410 (IQR, 71 337–183 452) in the nitrite group and 176 492 (IQR, 89 831–245 094) in the control group (*P*=0.16; Figure 3B).

In the 9 patients with TIMI >1 flow at time of infusion, baseline characteristics were similar between the groups aside from a significantly longer ischemia time in the nitrite group (Online Table II). No evidence of a difference in infarct size assessed by CK or troponin T AUC was seen in the patients with TIMI flow >1 treated with nitrite compared with placebo, although there was a trend to increased values in the nitrite group (Online Table II).

In the TIMI flow ≤1 group, there was a significant decrease in CMR-determined myocardial infarct size (15.31 [12.36–18.27] versus 20.08 [16.72–23.43]; *P*=0.03) associated with an increased myocardial salvage index (0.56 [0.50–0.62] versus 0.43 [0.37–0.49]; *P*=0.002; Figure 2B) associated with a reduction in MVO (37% versus 72.4%) in the nitrite-treated patients (Table 3). No evidence of difference in infarct size or AAR was seen in patients with TIMI flow >1 treated with nitrite compared with placebo (Table 3).

Safety and Tolerability of Nitrite

After administration of nitrite, 45% of patients developed a >10% decrease in systolic blood pressure (within 10 minutes); however, the magnitude and incidence was similar to the control group and did not alter clinical management. There was a small (but clinically insignificant) rise in met-Hb in the patients receiving nitrite; however, the levels were not different to the control group (Table 2).

During the first 48 hours after reperfusion, 7 adverse clinical events were recorded in the control group compared with 3 in the nitrite group (Table 2). One year after infarction, 6 MACE events were recorded in the control group compared with 1 in the nitrite group (*P*=0.04; see Table 2). There were no differences in the prescription of prognostic medication between the treatment groups at discharge or at 1 year of follow-up (Table 2).

Plasma NOx Levels

Similar nitrate and nitrate levels between the groups were evident at baseline, but an increase in circulating nitrite, but not nitrate, levels was evident at 30 minutes after sodium nitrite administration, indicating successful administration (Figure 4).

Platelet Reactivity

Platelet aggregation and P-selectin expression changed substantially over time (Figure 5 and Online Figure III and IV) in both groups. In all conditions, platelet reactivity was greatest at baseline with no differences between the treatment groups in the whole cohort (eg, unstimulated mean±SD P-selectin expression

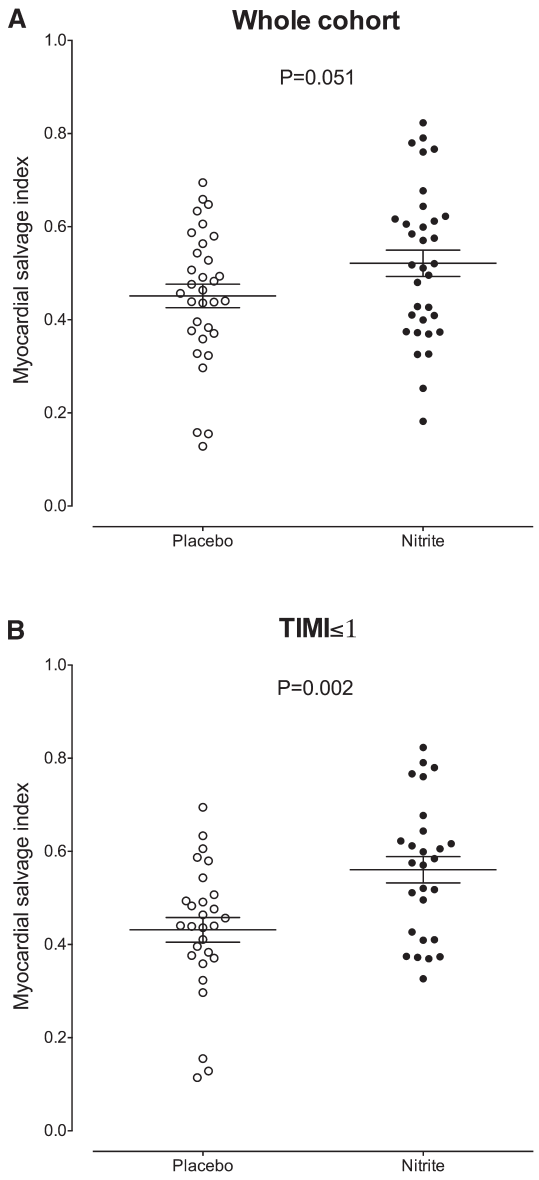

Figure 2. Effect of intracoronary nitrite on cardiac MRI (CMR)-determined myocardial salvage index. A, The myocardial salvage index on CMR is presented for 35 thrombolysis in myocardial infarction (TIMI) flow=0 to 3 patients in the control and 33 patients in the nitrite group. **B**, Myocardial salvage index is reduced in the nitrite-treated group of 27 TIMI≤1 patients vs 28 in the control. Significance evaluated using unpaired *t* test, and data shown as mean±SEM.

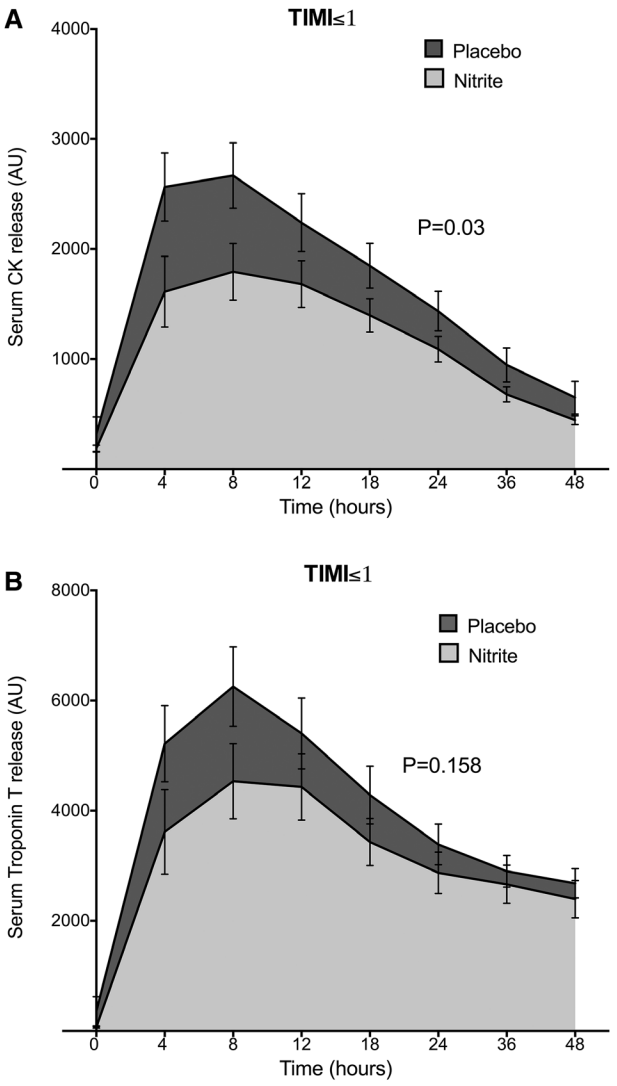

Figure 3. Intracoronary nitrite lowers infarct size by biomarker assessment in the thrombolysis in myocardial infarction (TIMI) flow ≤1 subgroup. Serum creatine kinase (CK) was measured at baseline and between 4 to 48 hours after coronary reperfusion. Curves for the nitrite and control group are shown in **A**. Serum troponin T was measured at the same time points as CK and curves shown in **B**. T bars denote standard errors of the mean (SEM).

in the whole cohort was 8.9±4.6% and 7.9±4.8%, and ADP-induced P-selectin expression was 47.2±18.9 and 46.9±18.2 in the placebo and nitrite-treated groups, respectively). In both groups, there was a decrease at 4 hours, followed by a slight elevation at 24 hours and a further decrease by 6 months. However, these changes postbaseline were all suppressed in the nitrite group versus placebo in both the whole cohort ($P<0.05$ or 0.01; Figure 5, Online Figure III and IV) and in the TIMI ≤1 subgroup ($P<0.01$, Figure 5, Online Figure III and IV). Post hoc analyses demonstrated that the reactivity of platelets to activating stimuli (only ADP shown for clarity) seems directly associated to CMR-determined infarct size (Figure 5) with 6 month CMR infarct size positively associated with both P-selectin expression ($r=0.401$, $P=0.002$; Figure 5E) and platelet aggregation ($r=0.344$, $P=0.007$; Figure 5F) at 6 months in response to ADP.

Discussion

In this proof-of-concept phase 2 study, intracoronary administration of nitrite at the time of reperfusion in patients with AMI was not associated with a reduction compared with placebo in the primary outcome measure of infarct size as assessed by cardiac biomarkers. There was greater myocardial salvage index with an 18% increase in the nitrite group compared with placebo (this was on the boundaries of conventional statistical significance ($P=0.05$)) and significant reductions in MACE at 6 months and 1 year. In a single retrospective subgroup analysis of patients with TIMI flow ≤1, at the time of primary PCI, treatment with nitrite was associated with a 20% reduction in infarct size compared with placebo as assessed by cardiac biomarkers (AUC for CK).

Table 3. CMR Data for Study Population Split by TIMI Flow at Presentation

	Whole Cohort			TIMI ≤1			TIMI >1		
	Nitrite (n=33)	Placebo (n=35)	*P* Value	Nitrite (n=27)	Placebo (n=30)	*P* Value	Nitrite (n=4)	Placebo (n=5)	*P* Value
Baseline CMR									
LVEDVi, mL/m^2	76.13 (71.11–81.14)	70.58 (65.22–75.94)	0.13	75.69 (69.93–81.44)	70.33 (64.31–76.34)	0.19	78.01 (51.65–104.40)	71.99 (54.30–89.68)	0.58
LVESVi, mL/m^2	36.16 (32.56–39.75)	35.71 (30.94–40.48)	0.88	35.16 (31.20–39.12)	35.41 (30.62–40.20)	0.94	39.90 (18.85–60.94)	37.37 (12.14–62.80)	0.84
LVMi, g/m^2	63.02 (58.65–67.40)	58.23 (54.67–61.79)	0.09	61.07 (56.27–65.88)	58.07 (54.44–61.71)	0.31	74.11 (58.19–90.03)	59.12 (40.75–77.49)	0.13
LVEF, %	52.87 (49.88–55.86)	50.07 (46.46–53.67)	0.23	53.86 (50.40–57.32)	50.00 (46.43–53.57)	0.12	49.55 (40.32–58.78)	50.50 (29.96–71.04)	0.92
IS, % LV	17.10 (14.12–20.08)	19.55 (16.40–22.70)	0.25	15.31 (12.36–18.27)	20.08 (16.72–23.43)	0.03	21.43 (9.62–33.23)	15.86 (0.42–31.30)	0.40
AAR, % LV	34.58 (31.62–37.55)	33.05 (29.40–36.72)	0.52	33.89 (30.54–37.24)	33.27 (29.12–37.42)	0.82	35.71 (21.15–50.28)	31.74 (21.62–41.87)	0.51
MSI	0.52 (0.46–0.58)	0.44 (0.39–0.49)	0.05	0.56 (0.50–0.62)	0.43 (0.37–0.49)	0.002	0.41 (0.31–0.51)	0.54 (0.30–0.78)	0.17
MVO									
No., %	16 (48.5%)	23 (69.7%)	0.13	10 (37.0%)	21 (72.4%)	0.02	3 (75.0%)	1 (25.0%)	0.14
Amount, g (median IQR)	2.98 (0–6.25)	3.47 (0–4.75)	0.34	1.00 (0.80–5.87)	4.50 (1–7.50)	0.003	1 (0–8.25)	1 (0–1)	0.09
6 mo CMR	(n=29)	(n=33)		(n=25)	(n=29)		(n=2)	(n=4)	
LVEDVi, mL/m^2	82.13 (74.80–89.46)	75.62 (69.90–81.34)	0.15	79.27 (72.01–86.54)	75.37 (69.20–81.53)	0.40	109.94 (–184.6–404.5)	80.57 (60.05–101.1)	0.16
LVESVi, mL/m^2	36.50 (31.48–41.52)	34.85 (30.53–39.17)	0.61	33.80 (30.08–37.54)	34.94 (30.16–39.73)	0.71	64.97 (–216.3–346.2)	37.42 (25.62–49.21)	0.13
LVMi, g/m^2	55.67 (51.66–59.68)	51.20 (48.17–54.24)	0.07	54.21 (50.35–58.07)	51.09 (47.75–54.44)	0.21	73.36 (–80.60–227.3)	52.14 (46.98–57.30)	0.05
LVEF, %	55.93 (52.71–59.15)	54.75 (51.62–57.87)	0.59	57.19 (54.12–60.27)	54.51 (50.96–58.05)	0.25	42.43 (–61.45–146.30)	53.51 (43.38–63.65)	0.18
IS, %	11.88 (9.52–14.24)	13.15 (10.75–15.56)	0.45	10.69 (8.38–13.02)	13.70 (11.16–16.24)	0.08	16.33 (–9.40–42.06)	10.59 (0.24–20.93)	0.32

Values shown as mean (95% CI).

AAR indicates area at risk; CMR, cardiac MRI; LVEDVi, indexed left ventricle end-diastolic volume; LVEF, left ventricle ejection fraction; LVESVi, indexed left ventricle end-systolic volume; LVMi, indexed left ventricle mass; MSI, myocardial salvage index; MVO, microvascular obstruction; and TIMI, thrombolysis in myocardial infarction.

This effect was replicated by the CMR analyses, demonstrating a reduction of infarct size of 25% associated with a greater myocardial salvage index and reduced platelet reactivity.

In this study, we show statistically significant reductions of infarct size in the subgroup of patients with TIMI flow ≤1, but not in the whole cohort. Important determinants of infarct size after primary PCI include AAR and the duration of ischemia,[27] both of which may be confounding variables in the present study. Despite the use of best practice through randomization and double blinding, nitrite-treated patients had a longer mean ischemia time compared with the placebo-treated group, which is known to adversely affect myocardial salvage and infarct size.[28,29] Importantly, in the subgroup analysis in those with TIMI flow ≤1, there were no differences in ischemia time or any other baseline values between the groups. This result suggests that, for nitrite to be most effective in reducing infarct size in STEMI patients, it needs to be administered whilst the culprit artery is still occluded. The mean ischemia time in the whole cohort (≈189 minutes) reflects well when compared with other studies assessing potential cardioprotective strategies, for example, cyclosporine[8] and postconditioning[6,26] with values ranging from 331 to 252 minutes. The comparatively reduced ischemia time in the present study likely underlies the smaller infarct sizes seen herein in comparison to other published studies (eg, Refs 6 and 8), a fact corroborated by the CMR analyses. It is noteworthy that, although not statistically different, the AAR assessed by angiographic scores displayed a trend to be larger in the nitrite-treated group in the whole cohort with increases of 5% to 14% depending on the method used: this could confound the results with theoretically larger infarcts in the nitrite group.

A recent study using intravenous nitrite in STEMI patients, with a prespecified recruitment criteria of TIMI flow ≤1 before reperfusion, showed no reduction in infarct size.[30] These findings contrast directly with our subgroup assessment of TIMI flow ≤1 patients, where a substantial cardioprotective effect, in almost all measures of cardioprotection, was evident. This difference may relate to differences in the route of administration and dose. In the Frenneaux study, nitrite was administered intravenously using a dose shown previously to achieve circulating levels of 6 μmol/L, in dogs, that was associated with profound cardioprotection.[18] This concentration sits within the previously demonstrated efficacious levels of 3 to 12 μmol/L

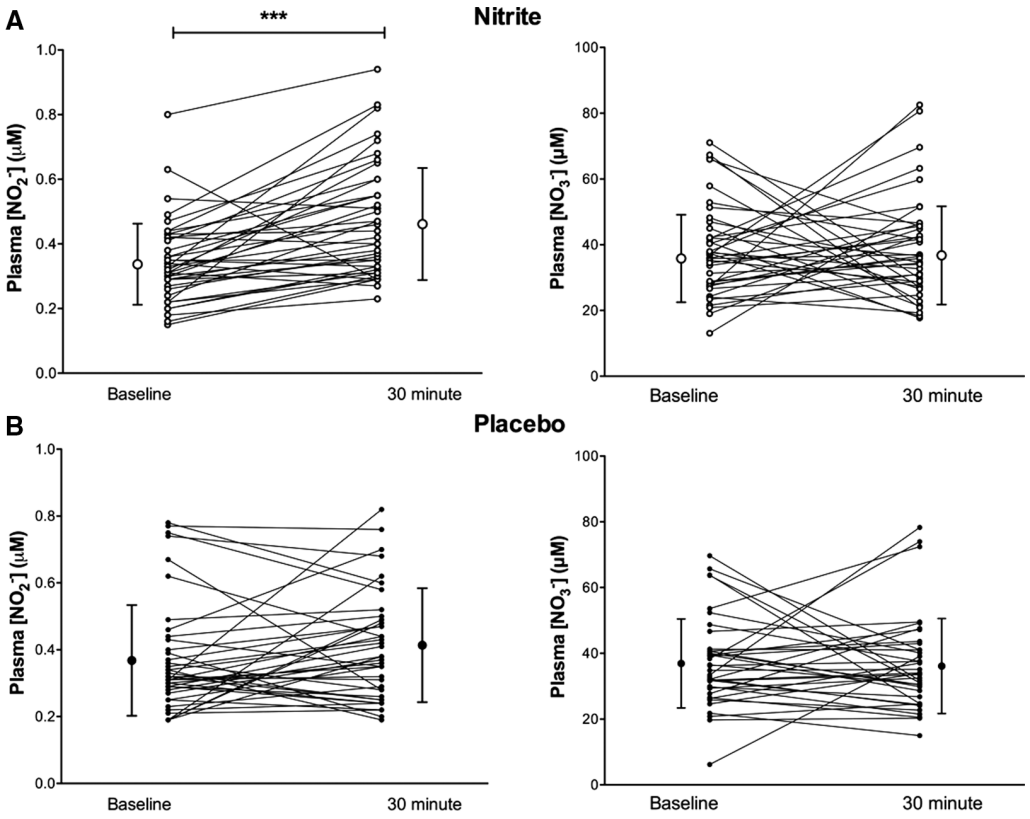

Figure 4. Plasma NO_2^- and NO_3^- levels pre and post intervention. Plasma NO_2^- and NO_3^- levels measured at baseline and 30 minutes after delivery of either intracoronary nitrite or placebo in all patients. Each line representing a single patient and the difference between baseline and 30 minute plasma NO_2^- and NO_3^- levels shown for each patient in the nitrite group (**A**) and placebo (**B**). Error bars represent mean±SD for each group. ***P<0.0001 using paired t test (NO_2^-, nitrite; NO_3^-, nitrate).

in numerous preclinical studies in vivo in various species.[15–17] Unfortunately, in the Frenneaux study,[30] this dose increased circulating levels of nitrite from 0.76 to 1.4 μmol/L only, suggesting that the pharmacokinetics of intravenously administered nitrite in humans is different from dogs. In our study, we gave a bolus dose of nitrite directly into the coronary artery, providing a local estimated concentration of ≈10 μmol/L and at the least 3 μmol/L. We think that achieving this high local concentration before reperfusion is the key factor underlying the efficacy in the TIMI flow ≤1 patients in our cohort.

In patients with significant coronary flow (TIMI flow>1) before infusion, a lack of benefit is not unexpected, although the numbers are small. Extensive preclinical evidence demonstrates that the cytoprotective properties of nitrite in models of myocardial infarction[14–18] is most evident with application into or on the ischemic organ, with the culprit vessel occluded at time of drug delivery. Bioactivation of nitrite to nitric oxide within the circulation does occur under physiological conditions in humans[20,22]; however, this phenomenon is enhanced with decreasing oxygen tension underlying its improved bioactivity under hypoxic/ischemic conditions.[11,22] Standard care pathways for STEMI patients presenting to hospitals for primary PCI include early and efficient administration of antiplatelet and antithrombotic therapies. Indeed, studies suggest that >40% of patients will have spontaneously reperfused in the infarct-related territory, resulting in significant coronary flow (TIMI flow >1) within the culprit coronary artery before revascularization.[31] Assessing whether nitrite would benefit, or indeed cause no harm, to a representative group of patients, including those with TIMI flow >1, is important.

The mechanisms underlying the beneficial effects of nitrite have been attributed to its conversion to nitric oxide, which improves mitochondrial function but also exerts anti-inflammatory and antiplatelet effects.[12,13] Interestingly, MVO was reduced in patients treated with nitrite. MVO has been implicated in worse clinical outcomes because of poor myocardial perfusion, despite epicardial coronary artery revascularization.[32] In our study, the incidence of MVO at the initial CMR scan was reduced by ≈20% and 35% in the nitrite-treated group in the whole cohort and the TIMI flow <1 subgroup, respectively. It is worth noting that factors that affect MVO, such as comorbid conditions and the use of antiplatelet and anticoagulant therapy, were similar between the 2 treatment groups. In addition, although statistical differences were shown with 2 group statistical comparisons we did not perform statistical correction for multiple comparisons in this study. Thus, further prospective studies powered for statistical significance across all of the CMR-related measures would be required to confirm the validity of our observations.

Our exploratory mechanistic analyses assessing platelet function suggest that the underlying reason for the difference in MVO may relate to reductions in platelet reactivity. Post hoc analyses show direct correlation between platelet reactivity and infarct size, and we suspect that the reduced infarct size and, as a consequence, a reduced systemic inflammation might underlie this improvement with nitrite. Further analyses

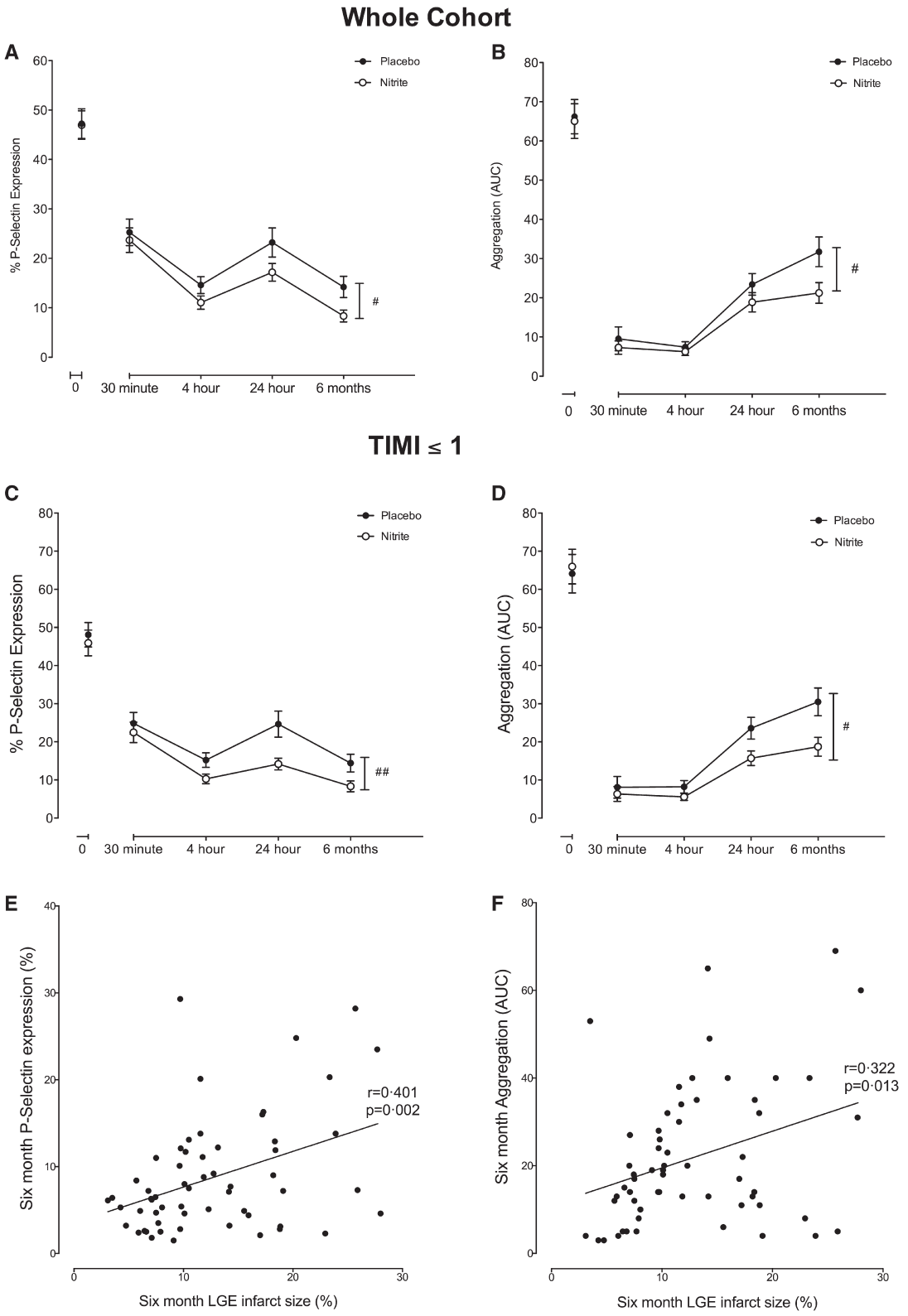

Figure 5. Platelet reactivity post intervention. Platelet reactivity measured at baseline, 30 minutes, 4 hours, 24 hours, and 6 months after coronary reperfusion. Platelet P-selectin expression assessed in whole blood in response to adenosine diphosphate (ADP; 10 μmol/L) is shown for nitrite versus placebo for all patients (**A**). **B**, Whole blood impedancce aggregometry in response to the same ADP stimulus in all patients. **C**, P-selectin expression in response to ADP in patients with thrombolysis in myocardial infarction (TIMI) flow <1. **D**, Aggregation in response to ADP in the TIMI <1 subgroup. All panels show nitrite-treated versus placebo. Data expressed as mean±SEM. #*P*<0.05, ##*P*<0.01 for 2-way repeated measures ANOVA. **E**, There was a positive association between platelet P-selectin expression in response to ADP and late gadolinium enhancement (LGE) assessed infarct size on cardiac MRI (CMR) at 6 months. **F**, A similar positive association between platelet aggregation in response to ADP and LGE CMR infarct size at 6 months. Correlations determined using Pearson's correlation coefficient.

assessing levels of systemic inflammation and whether they correlate with infarct size/platelet reactivity are additional secondary outcome measures that should inform on this possibility. However, further prospective studies powered for assessment of platelet reactivity are warranted.

Although small, this study also shows no indication that intracoronary nitrite administration has adverse effects in the cohort as a whole or within the subgroup. Specifically, we assessed blood pressure as a result of the known vasodilator and blood pressure lowering effects[21] of raised circulating nitrite levels. The data demonstrate that blood pressure did drop in some patients, although this was equally evident in both arms and likely caused by bradycardia and hypotension, which are a common feature of reperfusing occluded coronary arteries (Bezold–Jarisch reflex), particularly the right coronary artery. We also assessed methemoglobinemia because of the known interaction of nitrite with oxyhemoglobin to generate methemoglobin, particularly occurring with systemic nitrite infusions,[33] and here also no adverse effect was noted. Preclinical evidence indicates that nitrite is cytoprotective against ischemia–reperfusion injury only when given at concentrations (3–12 μmol/L) that far exceed physiological (0.1–0.4 μmol/L) levels. We suggest that the lack of adverse effect, despite the use of high (supraphysiological) levels of nitrite in this study, reflects the advantage of intracoronary nitrite administration, that is, achieving high local concentrations within the myocardium only. An additional advantage of the intracoronary route is that it provides an option causing no delay in reperfusion. This is compared with other therapies, including cyclosporine and exenatide, where intravenous administration may result in both greater side-effects and a delay in reperfusion when administered.

Study Limitations

Despite the same method of drug delivery used in both randomized patient groups, there was a longer ischemia time in the nitrite group, which will have limited the potential effect of the therapy seen in the study cohort. The study was powered based on the enrolment of all-comers to prevent any treatment delay and to test the therapy in as broad a group as possible. Despite this, sufficient numbers of patients with TIMI flow ≤1 were available to conduct powered statistical analyses.

Further studies powered for assessing safety in TIMI flow >1 patients are essential to determine the generalized safety of intracoronary nitrite administration in patients presenting with an AMI and could be incorporated into a larger phase 3 study assessing the therapeutic utility of nitrite in AMI.

For the CMR measures, our study was powered for single statistical comparison for the secondary outcome measure of infarct size; we did not conduct multiple testing. However, CMR provides information regarding several other features of cardiac structure and function as detailed in the tables. A further study powered sufficiently for statistical comparison of multiple CMR-derived measures of infarct size, MVO, and other indices providing valuable information regarding cardiac function, such as ejection fraction, may be of value to confirm the observations in this study.

Infarct size is an intermediate outcome measure that is commonly used to assess cardioprotective strategies in STEMI patients. However, as an intermediate outcome measure, this does not provide clear understanding on hard outcomes, such as MACE. Our study was not powered to detect changes in MACE, and although we saw evidence of benefit, the low number of events prevent drawing of any reliable conclusion. We suggest that our data provide strong support for conducting a phase 3 study in patients with TIMI ≤1 flow at point of revascularization, assessing the therapeutic potential of intracoronary nitrite administration with MACE as the primary outcome measure.

Conclusion

This study demonstrates that intracoronary nitrite infusion should be added to the list of promising cardioprotective agents for potential use in AMI when administered intracoronary at the point of revascularization. Further investigation of this potential in a larger Phase 3 clinical trial is warranted.

Acknowledgments

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Disclosures

A. Ahluwalia is a director of Heartbeet Ltd. The other authors report no conflicts.

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Novelty and Significance

What Is Known?

- Primary percutaneous coronary intervention (PCI) is used currently for the treatment of acute myocardial infarction, but significant morbidity and mortality rates remain, in part as a result of the damaging effects of reperfusion after revascularization.
- Reducing reperfusion injury could improve outcomes.
- Extensive preclinical data suggest that local delivery of sodium nitrite reduces reperfusion injury.

What New Information Does This Article Contribute?

- This phase 2 double-blind randomized placebo controlled clinical trial assessed the efficacy of intracoronary nitrite infusion during primary PCI post acute myocardial infarctions.
- There was a significant reduction in infarct size in a subgroup of patients with occluded culprit arteries at the time of PCI.
- This effect was associated with an apparent decrease in platelet reactivity over the 6 months after PCI and a reduction in major adverse cardiovascular events at 6 months and 1 year.

The results of this clinical trial demonstrate that local intracoronary administration of sodium nitrite reduces infarct size as assessed by the measurement of cardiac enzyme release and scar size determined using cardiac MRI in patients with an occluded artery (thrombolysis in myocardial infarction flow ≤1) at the time of PCI. The intracoronary administration of nitrite before balloon inflation offers a potential cardioprotective strategy that causes no significant delay in delivery of the primary angioplasty procedure. In addition, this procedure enables high dose delivery of nitrite that is not associated with significant methemoglobinemia or a decrease in blood pressure, the 2 potential concerns with nitrite delivery. The results of this study suggest that intracoronary nitrite administration to the culprit vessel of select patients presenting with acute myocardial infarctions has no adverse safety profile and may provide a new therapeutic option as an adjunct to primary angioplasty. These findings warrant further investigation in larger outcome studies.

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Randomized Phase 2 Trial of Intracoronary Nitrite During Acute Myocardial Infarction

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Correction

In the *Circulation Research* article by Jones et al (Randomized Phase 2 Trial of Intracoronary Nitrite During Acute Myocardial Infarction. *Circ Res*. 2015:116:437-447. doi: 10.1161/CIRCRESAHA.116.305082.), the authors recently discovered an error in their Abstract that should be corrected as follows: In contrast, there was an improvement in myocardial salvage index (P=0.05) and reduction in major adverse cardiac event at 1 year (2.6% versus 15.8%; P=0.04) in the nitrite group.

The authors apologize for this error, and the error has been noted and corrected in the online version of the article, which is available at http://circres.ahajournals.org/content/116/3/437.full.

(*Circ Res*. 2015;116:e55. DOI: 10.1161/RES.0000000000000051.)

Supplemental material

Randomised phase 2 trial of intra-coronary nitrite during acute myocardial infarction

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Supplemental Methods

Informed consent

Seeking informed consent for clinical research from patients suffering acute myocardial infarction (AMI) is an ethical challenge owing to the medical condition of the patients, the emergency situation, and the limited time available. There was no guaranteed solution to the particular difficulties of informed consent in this situation. It is extremely important that patients are provided with information, which is as concise and simple as possible, although sufficient for them to make an informed decision. To achieve this we firstly excluded patients who were unconscious, critically unstable (cardiogenic shock) or deemed unable to consent (pain, distress, language) and then followed a two step process of consent that was based upon our extensive investigations to identify the most efficient and considerate mechanism of gaining consent from patients in this emergency setting. A clear concise study summary sheet was shown to the patient during the consent process, this was a one-page sheet with diagrams, clearly explaining the procedure and events. A more detailed patient information sheet (PIS) was given following the procedure for reading. Secondly the oral information provided during the consent process had been discussed and planned with members of the public who agreed that they explain the trial effectively. There was a clear algorithm to follow with rehearsed statements. These were designed to match the summary PIS. Patients then signed an approved consent form in addition to the standard primary PCI consent form. This process and all forms were approved by the local research ethics committee.

Thrombolysis In Myocardial Infarction (TIMI)

Coronary angiograms obtained before and after primary PCI were reviewed by two experienced observers blinded to treatment allocation and clinical data. From these angiograms an assessment of TIMI flow grade and AAR using standard (BARI and APPROACH) validated approaches (see online supplement for further detail). both the modified Bypass Angioplasty Revascularisation Investigation [BARI][1, 2] and modified Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease [APPROACH][3] jeopardy scores were made.

Cardiac imaging protocols

Cardiac magnetic resonance (CMR) imaging was performed on a 1·5 T Philips Achieva scanner with a cardiac 32-channel phased array coil. Balanced steady-state free precession cine imaging was used to acquire 10-12 short axis slices (8 mm slice thickness, 2mm gap) with one slice per breath-hold. Sequence parameters were 1.5 ms echo time (TE), 3.1 ms repetition time (TR), and acquired voxel size was 1.8 x 1.86mm with a typical FOV of 360mm in the phase encode direction. We acquired 45 phases with 25% phase sharing. Parallel imaging (SENSE) was used with an acceleration factor of 2.0.

Delayed enhancement images were acquired ten minutes after injection of a dose of 0·2 mmol/kg of gadoterate meglumine (Dotarem) for late gadolinium enhancement. A T1-weighted segmented inversion-recovery gradient echo pulse sequence (TR 3.9ms TE 1.9ms, flip angle 15^{o}, voxel size of 2 x 2mm, typical FOV 360mm) was used to obtain 10-12 short axis slices (matched with short-axis cine images) with one slice per breath-hold. The inversion time was adjusted individually according to a T1 scout sequence (Look-Locker). Images were acquired every other heart beat with 2 signal averages.

Myocardial oedema was assessed using fat suppressed T2-weighted triple inversion turbo spin echo STIR (Short tau inversion recovery) imaging (TE 80ms, TR 2 heart beats, TSE factor 31, voxel size 1.8 x1.8mm). 10-12 slices were obtained (8mm per slice, 2mm gap matched to DE/cine slices) with one slice per breath-hold. This sequence has previously been used and validated for assessment of myocardial oedema and MSI[4-7].

Images were anonymised, batched and analyzed in blinded fashion by two experienced operators. Scar and oedema volumes were calculated by manually drawing endocardial and epicardial contours followed by semi-automated selection of normal remote myocardium per slice. Myocardial oedema was described as >2SD in signal intensity from remote normal myocardium. Infarct size was calculated using the full-width half maximum method as previously described[8]. In case of discordance between operators, blinded review by a level III accredited CMR reader was

performed. Analysis was performed using dedicated software (CVI[42], Circle Cardiovascular Imaging Inc, Calgary, Alberta, Canada). Interobserver variability was calculated.

Platelet reactivity

Platelet reactivity was assessed by determining platelet aggregation and P-selectin expression at baseline, 30 minutes post delivery of nitrite/placebo, 4, 24 hours and 6 months after infarction. These were assessed in whole blood in response to adenosine diphosphate (ADP) (10 μmol/L), collagen (3 μg/mL), and PBS (as control) using an impedance aggregometer (MultiplateRanalyzer, Dyabyte Medical, Germany) and flow cytometry respectively using previously published protocols[9]. These measures were conducted at baseline, 30 minutes post delivery of nitrite/placebo, 4, 24 hours and 6 months after infarction.

Whole-blood aggregometry

Platelet aggregation was assessed in whole blood in response to ADP (10μmol/L), collagen (3μg/ml) and PBS (as control) using an impedance aggregometer (Multiplate Ranalyzer, Dyabyte Medical, Germany) measured over a six-minute period. Aggregation is quantified as AUC giving a measure of total resistance Ω^{*}time. Briefly 300mL of citrated whole blood was added to 300 mL of normal saline with 3 mmol/L$CaCl_2$ (Sigma, UK) and equilibrated with constant magnetic stirring for three minutes prior to the addition of agonist and platelet aggregation measurement.

P-Selectin expression

Two-colour whole blood flow cytometry was used to measure platelet P-selectin using a previously published protocol[10]. Whole blood was collected from individuals at the specified time-points. The samples were immediately incubated with selective antibodies, at room temperature for 20 minutes, and then fixed using 1% paraformaldehyde (Sigma,UK) stored at 4^{o}C and then analysed using a Becton Dickinson FACS Calibur flow cytometer (Becton Dickinson, SanJose, CA). The platelet population was identified preliminarily based on forward and side-scatter

properties, then further delineated via labeling with CD42b monoclonal antibody conjugated to allophycocyanin (APC). Gates were used to isolate this population, and CD62 (P-selectin) monoclonal antibody conjugated to (fluorosce-nisothiocyanate) FITC was used to determine P-selectin expression. Populations were further confirmed by use of antibody negative iso-types to P-selectin and CD42b.10,000 platelets were acquired in the CD42b region, and results were expressed as the percentage of platelets positive for P-selectin.

Ozone chemiluminescence

Briefly, to determine total nitrate and nitrite levels (collectively termed [NOx]), samples were added to 0.1 mmol/L vanadium (III) chloride in 1 mmol/L hydrochloric acid refluxing at 95 °C under nitrogen. Nitrite concentrations were determined by addition of samples to 0.09 mmol/L potassium iodide in glacial acetic acid under nitrogen at room temperature. [Nitrate] were calculated by subtraction of [nitrite] from [NO*x*] as previously described[11].

Major adverse events

Major adverse events at 48 h included including death, heart failure, acute myocardial infarction, stroke, recurrent ischaemia, need for repeat revascularization, renal/hepatic insufficiency, vascular complications, and bleeding. Heart failure was defined as dyspnea (either new-onset or persisting) accompanied by both physical signs of heart failure (pulmonary crackles/rales, peripheral oedema, jugular venous distension, S3 gallop, radiological evidence of pulmonary oedema) and a need for increased heart failure therapy (diuretic or other oral heart failure therapies e.g. ACEi or mechanical/surgical intervention).

Supplemental References

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Supplemental Results

Tables

Online Table I. Baseline characteristics of the TIMI <1 subgroup

	Nitrite (n=33)	Placebo (n = 33)	P value
Age (yrs) (mean±SD)	57.30±11.29	56.94±13.48	0.90
Sex (male/female)	29/4	28/5	0.99
Diabetes mellitus	2 (6.1)	1 (3.0)	0.99
Body-mass index (kg/m^2) (mean±SD)[a]	29.27±5.30	29.06±5.17	0.87
Hypertension	16 (48.5%)	8 (24.2%)	0.07
Hypercholesterolaemia	13 (39.4%)	10 (30.3%)	0.61
Heart rate (mean±SD)	70.94±18.97	79.06±22.10	0.11
Systolic Blood pressure (mean±SD)	120.76±29.64	132.94±23.44	0.07
Ischaemia time (minutes) (mean±SD)[b]	194.45±69.05	168.63±69.94	0.11
Culprit Vessel			0.57
Left anterior descending	8 (24.2%)	9 (27.3%)	
Circumflex	3 (9.1%)	5 (12.5%)	
Right coronary	22 (66.7%)	19 (57.6%)	
Syntax score (mean±SD)	13.29±5.42	13.68±5.42	0.77
DES use	29 (87.9%)	26 (81.3%)	0.51
Angiographic AAR			
APPROACH (mean±SD)	30.72±7.75	26.94±10.73	0.11
BARI (mean±SD)	27.32±10.88	24.75±9.26	0.31
Treatment before PCI			
Morphine	22 (66.7)	29 (87.9)	0.08
Treatment at time of PCI			
Heparin	33 (100)	33 (100)	0.99
Aspirin	33 (100)	33 (100)	0.99
Clopidogrel/Prasugrel (no/no)	29/4	30/3	0.99
Glycoprotein IIb/IIIa inhibitor	33 (100)	33 (100)	0.99
ST segment resolution >70%	33 (100%)	28 (84.8%)	0.02

Values shown as number (%) unless otherwise stated

Abbreviations: PCI, percutaneous coronary intervention; TIMI, Thrombolysis in myocardial infarction; DES, drug-eluting stent.

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

[b] Ischaemia time determined from symptom to balloon times for each patient.

Online Table II. Baseline characteristics of the TIMI >1 subgroup

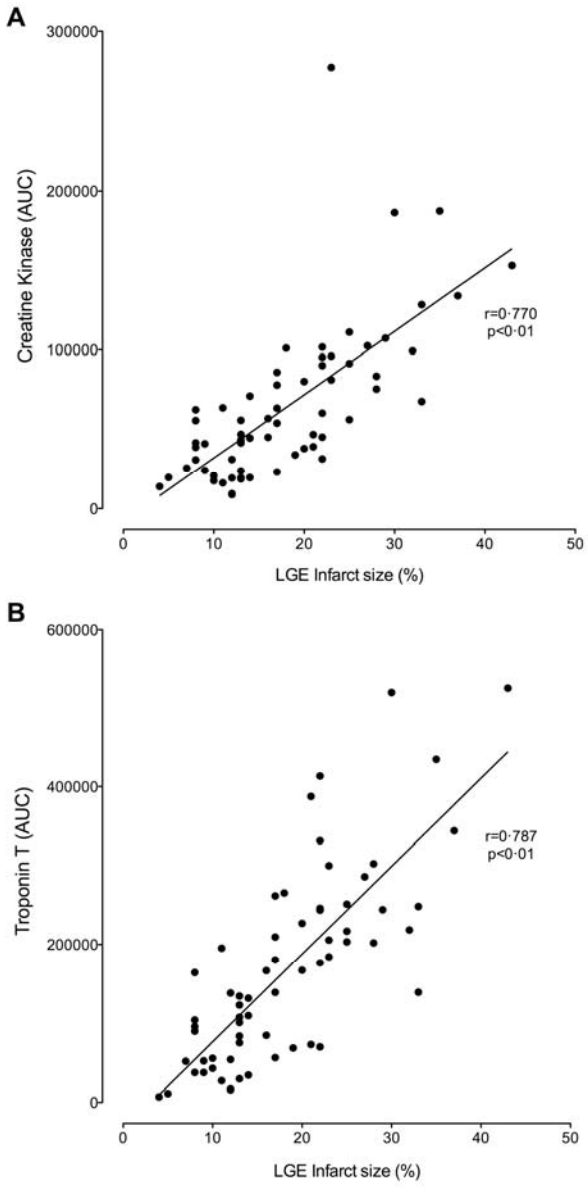
	Nitrite (n=4)	Placebo (n = 5)	P value
Age (yrs) (mean±SD)	54.75±5.50	60.00±13.87	0.50
Sex (male/female)	4/0	2/3	0.17
Diabetes mellitus	1 (25.0)	2 (40.0)	0.99
Body-mass index (kg/m^2) (mean±SD)[a]	28.53±5.43	25.95±5.85	0.54
Hypertension	3 (75.0%)	4 (80.0%)	0.99
Hypercholesterolaemia	3 (75.0%)	1 (25.0%)	0.21
Heart rate (mean±SD)	74.00±17.38	72.80±13.41	0.91
Systolic Blood pressure (mean±SD)	131.50±26.90	135.80±33.82	0.84
Ischaemia time (minutes) (mean±SD)[b]	286.80±96.70	174.33±55.19	0.04
Culprit Vessel			0.49
Left anterior descending	1 (25.0%)	2 (40.0%)	
Circumflex	1 (25.0%)	0 (0.0%)	
Right coronary	2 (50.0%)	3 (60.0%)	
Syntax score (mean±SD)	15.63±7.91	13.30±10.79	0.73
DES use	1 (25.0%)	2 (40.0%)	0.99
Angiographic AAR			
APPROACH (mean±SD)	31.68±10.15	25.18±9.98	0.30
BARI (mean±SD)	26.35±6.06	24.24±4.69	0.57
Treatment before PCI			
Morphine	3 (75.0)	4 (80.0)	0.99
Treatment at time of PCI			
Heparin	4 (100)	5 (100)	0.99
Aspirin	4 (100)	5 (100)	0.99
Clopidogrel/Prasugrel	3/1	5/0	0.44
Glycoprotein IIb/IIIa inhibitor	4 (100)	5 (100)	0.99
Infarct size (Median (IQR))			
Creatine Kinase	78398 (30945-104752)	21196 (13864-73887)	0.413
Troponin T	144798 (59452-207770)	83796 (55473-182372)	0.413

Values shown as number (%) unless otherwise stated

Abbreviations: PCI, percutaneous coronary intervention; TIMI, Thrombolysis in myocardial infarction; DES, drug-eluting stent.

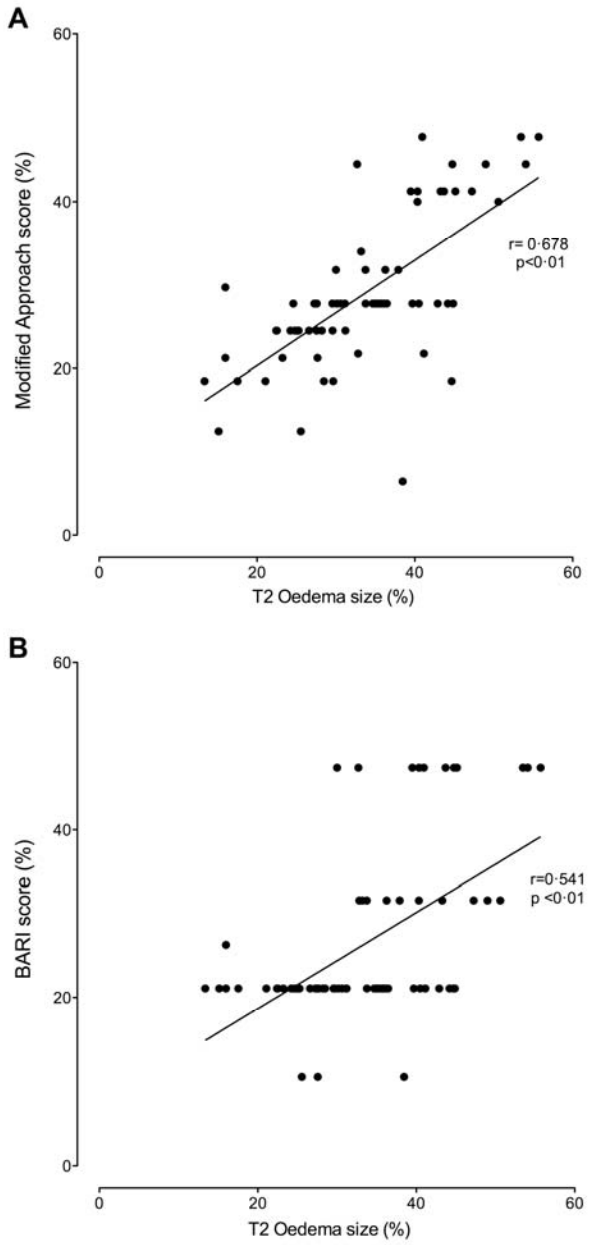
[a] The body-mass index is the weight in kilograms divided by the square of the height in meters.

[b] Ischaemia time determined from symptom to balloon times for each patient.



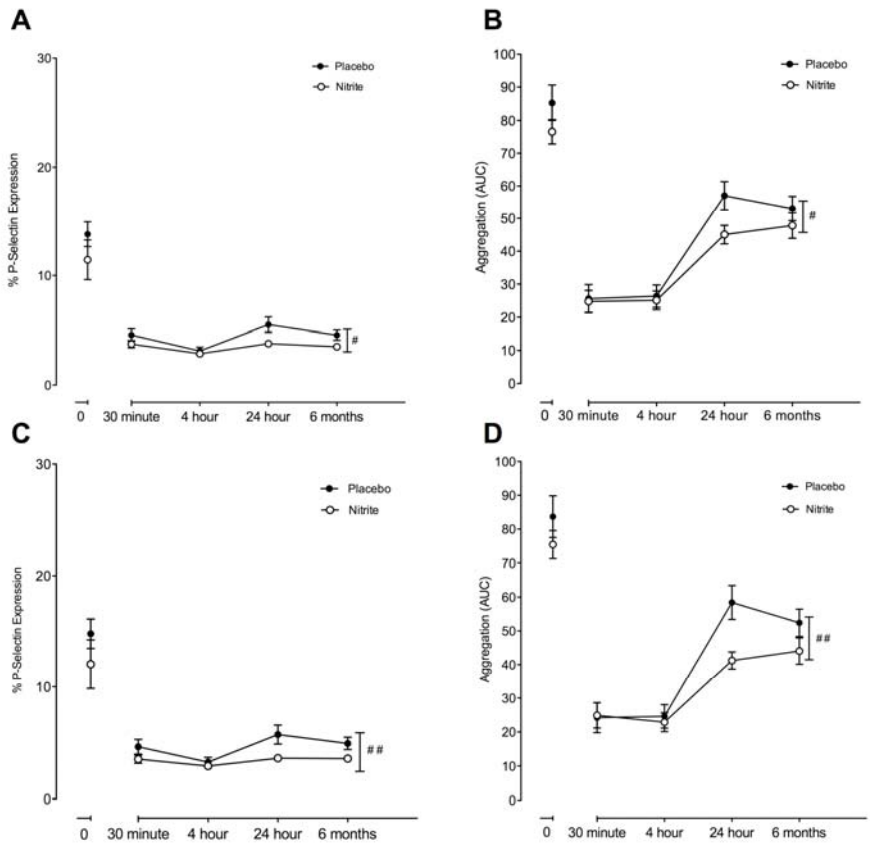
Online Figure I. Associations between cardiac biomarker assessment of infarct size and infarct size on cardiac magnetic resonance imaging

There was a significant positive correlation between infarct size assessed by creatine kinase area under the curve (AUC) and LGE (late gadolinium enhancement) assessed infarct size on CMR (late gadolinium enhancement) (r=0.770), as shown in panel A. Panel B depicts a similar positive association between troponin T AUC and LGE CMR infarct size (r=0.787). Associations determined using Pearson's correlation coefficient assessment.



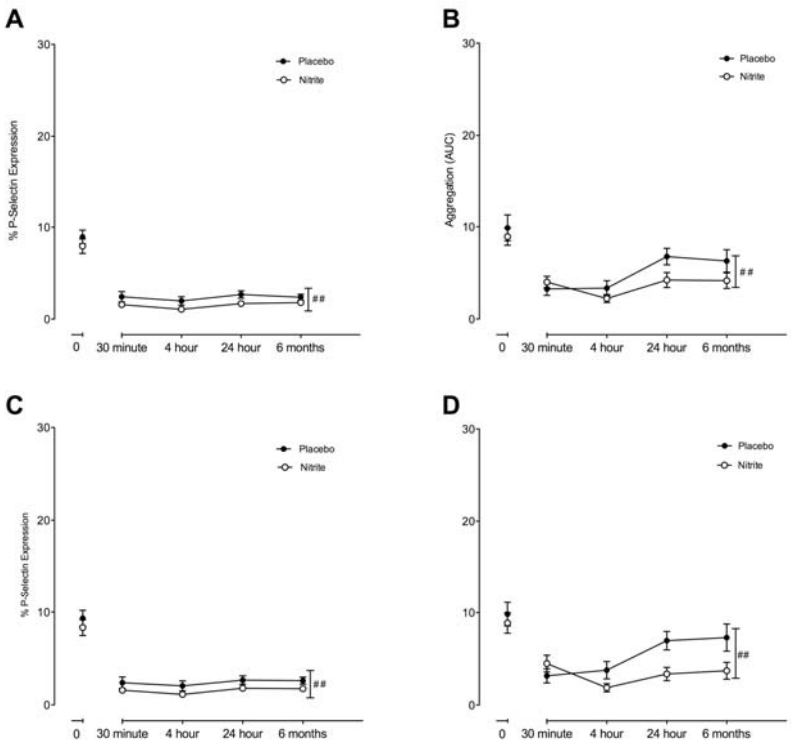
Online Figure II. Associations between angiographic area at risk scores and area at risk assessed by cardiac magnetic resonance imaging (CMR)

There was a significant positive correlation between the angiographic area at risk as assessed by the modified APPROACH score and the area at risk assessed by T2 oedema imaging on CMR (r=0.678), as shown in panel A. Panel B depicts a similar positive association between the modified BARI score and the area at risk assessed by T2 oedema imaging on CMR (r=0.541). Associations determined using Pearson's correlation coefficient assessment.



Online Figure III. Intracoronary nitrite lowers ex vivo assessed platelet reactivity to collagen

Platelet reactivity measured at baseline, 30 minutes, 4 hours, 24 hours and 6 months after coronary reperfusion. Platelet P-Selectin expression assessed in whole blood in response to collagen (3 μmol/L) is shown for nitrite versus placebo for all patients in panel A. Panel B show using whole blood impedance aggregometry in response to the same collagen stimulus in all patients. Panel C shows P-selectin expression in response to collagen in patients with TIMI flow <1. Panel D shows aggregation in response to collagen in the TIMI <1 subgroup. All panels show nitrite treated versus placebo. Data expressed as mean ± SEM. □ =P<0.05, □□=P<0.01, for two-way repeated measures ANOVA.



Online Figure IV. Intracoronary nitrite lowers baseline ex vivo assessed platelet reactivity

Platelet reactivity measured at baseline, 30 minutes, 4 hours, 24 hours and 6 months after coronary reperfusion. Platelet P-Selectin expression assessed in whole blood following incubation with phosphate buffered saline control (PBS) is shown for nitrite versus placebo for all patients in panel A. Panel B show whole, blood impedance aggregometry in response to the same PBS stimulus in all patients. Panel C shows P-selectin expression in response to PBS in patients with TIMI flow <1. Panel D shows aggregation in response to PBS in the TIMI <1 subgroup. All panels show nitrite treated versus placebo. Data expressed as mean ± SEM. □ =P<0.05, □□=P<0.01, for two-way repeated measures ANOVA. PBS=Phosphate buffered saline.