ORIGINAL ARTICLE

Intracoronary nitrite suppresses the inflammatory response following primary percutaneous coronary intervention

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ABSTRACT

Objective Recent work suggests that intracoronary nitrite reduces myocardial infarct size following primary percutaneous coronary intervention (PPCI) for acute myocardial infarction (AMI), although the exact mechanisms are unclear. We explored the effects of nitrite on reperfusion-induced inflammation, by assessing the levels of specific pro-inflammatory mediators, chemokines and adhesion molecules in plasma and circulating cell subtypes as exploratory end points in the NITRITE-AMI cohort.

Methods Peripheral blood leucocyte subsets, cell adhesion molecules, high-sensitivity C reactive protein (hs-CRP), the monocyte and neutrophil chemoattractants CCL2 and CXCL1, CXCL5, respectively were measured in the blood of patients who received either intracoronary sodium nitrite (N=40) or placebo (N=40) during PPCI for AMI. Major adverse cardiac events were recorded at 3 years post-PPCI.

Results In the placebo-treated patients, total circulating neutrophil numbers and levels of hs-CRP were raised postreperfusion and then decreased over time; in nitrite-treated patients these changes were suppressed compared with placebo up to 6 months post-PPCI ($p<0.01$). This effect was associated with reduced expression of neutrophil CD11b, plasma CXCL1, CXCL5 and CCL2 levels ($p<0.05$). There were no differences in the number of other any other leucocyte population measured (monocytes and lymphocytes) or activation markers expressed by these cells between the treatment groups. These effects were associated with a reduction in both microvascular obstruction and infarct size.

Conclusions Important reductions in neutrophil numbers and activation post-PPCI in patients with ST elevated myocardial infarction were associated with nitrite treatment, an effect we propose likely underlies, at least in part, the beneficial effects of nitrite upon infarct size.

Trial registration number NCT01584453.

INTRODUCTION

Inorganic nitrite is cardioprotective in preclinical models of acute myocardial infarction (AMI), reducing reperfusion injury and subsequent myocardial infarct size.[1 2] This research has been translated to the clinical setting with two separate clinical trials assessing the effect of sodium nitrite on infarct size in patients presenting with ST elevated myocardial infarction (STEMI), namely the Nitrite in Acute myocardial Infarction (NIAMI)[3] and NITRITE-AMI.[4] In this trial intra-coronary nitrite, when administered to patients with occluded arteries (ie, thrombolysis in myocaridial infarction[4] (TIMI) score 0–1), reduced infarct size. Interestingly, this effect of nitrite was associated with a reduction in major adverse cardiac events (MACE) at 1 year, although it should be noted that NITRITE-AMI was not powered for this outcome. The exact mechanisms by which this benefit was conferred are not clear.

Extensive preclinical assessments support the view that the protective activity of nitrite is entirely dependent upon its local conversion to nitric oxide (NO).[1 2] Mechanistically, the cytoprotective action of nitrite has been attributed, at least in part, to improvements of mitochondrial function via S-nitrosation of complex I[5] and inhibition of complex IV; effects resulting in reductions in reactive oxygen species generation, inhibition of opening of the mitochondrial transition pore and improved oxygen utilisation.[6] However, in addition to these effects nitrite also possesses other actions that might contribute to the potential beneficial effects in ischaemia/reperfusion (IR) injury, including reductions of platelet reactivity[7 8] and inflammatory cell recruitment.[9 10]

While the inflammation triggered as a consequence of ischaemia-induced cell death is a critical step in the reparative process following an AMI,[11] it is thought that the formation of platelet thrombi, endothelial protrusion[12] and inflammation[13] consequent to reperfusion contribute to reperfusion-induced microvascular obstruction and, as such, represents a potential tractable target.[11 14] We have demonstrated previously reductions in platelet reactivity following nitrite treatment in patients with STEMI; however, whether inflammatory pathways might also be reduced was not interrogated. To explore this possibility, we assessed the levels of specific pro-inflammatory components together with circulating cell subtypes as exploratory end points in the NITRITE-AMI cohort.[4]

METHODS

NITRITE-AMI study design and participants

NITRITE-AMI was a double-blind, randomised, single-centre, placebo-controlled trial to determine whether the intracoronary injection of sodium nitrite reduced infarct size in patients with STEMI undergoing PPCI.[4 15] The trial was approved by an independent ethics committee, the Medicines and

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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.[15] All volunteers gave written informed consent before being included in the study. After coronary angiography, patients were randomised (1:1) to a high-dose bolus injection of intracoronary sodium nitrite (1.8 µmol in 10 mL of 0.9% NaCl) or placebo (indistinguishable from the nitrite) (10 mL of 0.9% NaCl) administered via an over-the-wire balloon just prior to balloon inflation. All study personnel were blind to treatment allocation until all analyses had been completed.

Infarct size and MACE

Myocardial infarct size was assessed by 48-hour plasma creatine kinase (CK) and cardiac magnetic resonance imaging (CMR)[15] (see online supplementary material for details). At 3 years after AMI, MACE (defined as death, MI, recurrent revascularisation and heart failure) was recorded. All events were verified with source documentation.

Measurement of systemic inflammation

Blood was collected from 80 participants at baseline (ie, prior to PPCI), 30 min, 4 hours, 24 hours and 6 months post-PPCI (see online supplementary figure S1). Full blood counts were conducted according to Barts Health Trust protocols. Blood samples were also collected in citrate buffer and either used directly in flow cytometric analyses of cell subtype (44 patients; 23 nitrite and 21 placebo) and activation state or plasma generated for assessment of high-sensitivity C reactive protein (hs-CRP) or specific chemokine levels (72 patients; 34 nitrite and 38 placebo). Due to limitations regarding the volume of blood collection, not all measurements of inflammation were made at each timepoint. Total and differential cell counts were determined at baseline, 4 hours, 24 hours and 6 months. These timepoints reflect those shown previously to be associated with infarct size and outcome.[16–21]

Circulating inflammatory markers and chemokines

Flow cytometry was used for measurement of hs-CRP in plasma samples using eBioscience Flow Cytomix assays (eBioscience, UK) on a Becton Dickinson LSR Fortessa Cell Analyser (BD; flow cytometer) and analysed with BD FACSDiva software. The detection limits of the assay were 0.067 ng/mL, with mean inter-assay and intra-assay coefficients of variation for hs-CRP 3.4% and 9.6%, respectively.

Circulating plasma CXCL5 or CXCL1 levels were assessed using the Human CXCL5 DuoSet kit (DY254) or the Human CXCL1 DuoSet kit (DY275; R&D Systems, USA) as per the manufacturer's directions. A standard curve was generated with the provided standards and used to calculate the quantity of chemokine in the sample tested. All reactions were performed in duplicate, and the resulting values were averaged. The mean inter-assay and intra-assay coefficients of variation for CXCL5 and CXCL1 were 3% and 8.0% and 7%, respectively.

Leucocyte cell populations and their activation state

Citrated blood was incubated with specific antibodies to CD14, CD16, CD16b (BD Bioscience, UK, 20 µL) and CD3, CD4, CD8 (eBioscience, UK 2.5 µL) for cell subtype using flow cytometry as above (see online supplementary material for full details).

Data and statistical analysis

Analysis was performed using GraphPad Prism software V.5.0 for Mac OsX and SPSS V.19, (SPSS, Chicago, Illinois, USA). All p values were two sided and the border of significance was accepted as $p<0.05$. Analysis was based on the intention-to-treat principle. Baseline demographic and clinical variables were summarised for each arm of the study. Descriptive summaries of the distributions of continuous baseline variables were presented in terms of percentiles (eg, median, 25th and 75th percentile), while discrete variables were summarised in terms of frequencies and percentages. Comparisons were between the sodium nitrite-treated and placebo control-treated groups. Statistical analyses were conducted blind to the treatment groups. For comparisons between normally distributed data, statistical comparisons were

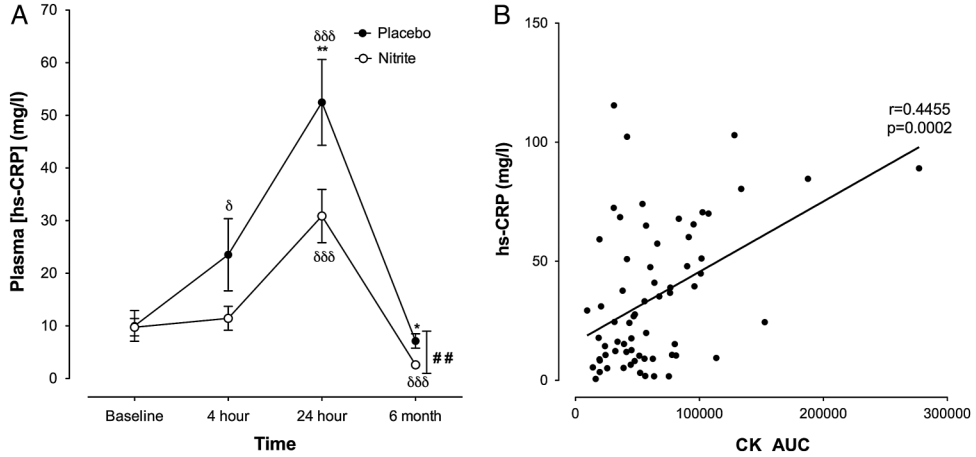

Figure 1 Effect of time and treatment on plasma high-sensitivity C reactive protein (hs-CRP). Serum levels of hs-CRP were measured at baseline, 30 min, 4 hours, 24 hours and 6 months after coronary reperfusion in a total of 79 patients (39 nitrite and 40 placebo). Data expressed as mean ±SEM. For comparisons of change relative to baseline within groups repeated measures one-way analysis of variance (ANOVA) followed by Dunnett's post-test was used with significance shown as δδδ for $p<0.001$ and δ for $*p<0.05$. For comparisons between treatments statistical significance is shown as ## for $p<0.01$ performed using two-way repeated measures ANOVA and $**p<0.01$, $*p<0.05$ for Bonferroni's post-test comparing specific timepoints. (B) Correlations determined using Pearson's correlation coefficient. The solid line represents the least-square fit of the data (CK, creatine kinase; AUC, area under the curve) between CK-determined infarct size and 24 hour plasma hs-CRP levels.

Table 1 Differential cell counts

	Placebo				Nitrite				
Cell count	Baseline	4 hours	24 hours	6 months	Baseline	4 hours	24 hours	6 months	Significance, p value
WBC ($\times 10^9$/mL)	12.8±3.7	13.0±3.6	10.8±3.0	8.1±2.1	12.6±3.6	11.3±3.0*	9.9±2.4	7.3±1.7	0.0079
Neutrophil	9.9±4.1	10.5±3.5	7.5±2.6	4.8±1.7	9.4±3.5	9.0±2.8	6.5±1.9	4.3±1.4	0.0062
Monocyte	0.8±0.4	0.7±0.4	0.9±0.4	0.6±0.2	0.7±0.3	0.7±0.3	0.9±0.3	0.7±0.2	0.4250
Lymphocyte	2.3±0.9	1.8±0.7	2.3±0.7	2.3±0.8	2.3±1.4	1.6±0.6	2.3±0.7	2.2±0.6	0.3656

Blood samples for differential cell count were collected at baseline, 4 and 24 hours and 6 months post-PPCI in 80 patients (40 nitrite, 40 placebo). Data expressed as mean±SD. Statistical analysis conducted using repeated measures two-way ANOVA with treatment group and time as the two factors, and p values indicated in the table. Bonferroni's post-test was conducted comparing specific timepoints between the nitrite and placebo groups and is shown as *p<0.05.
ANOVA, analysis of variance; PPCI, primary percutaneous coronary intervention; WBC, white blood cells.

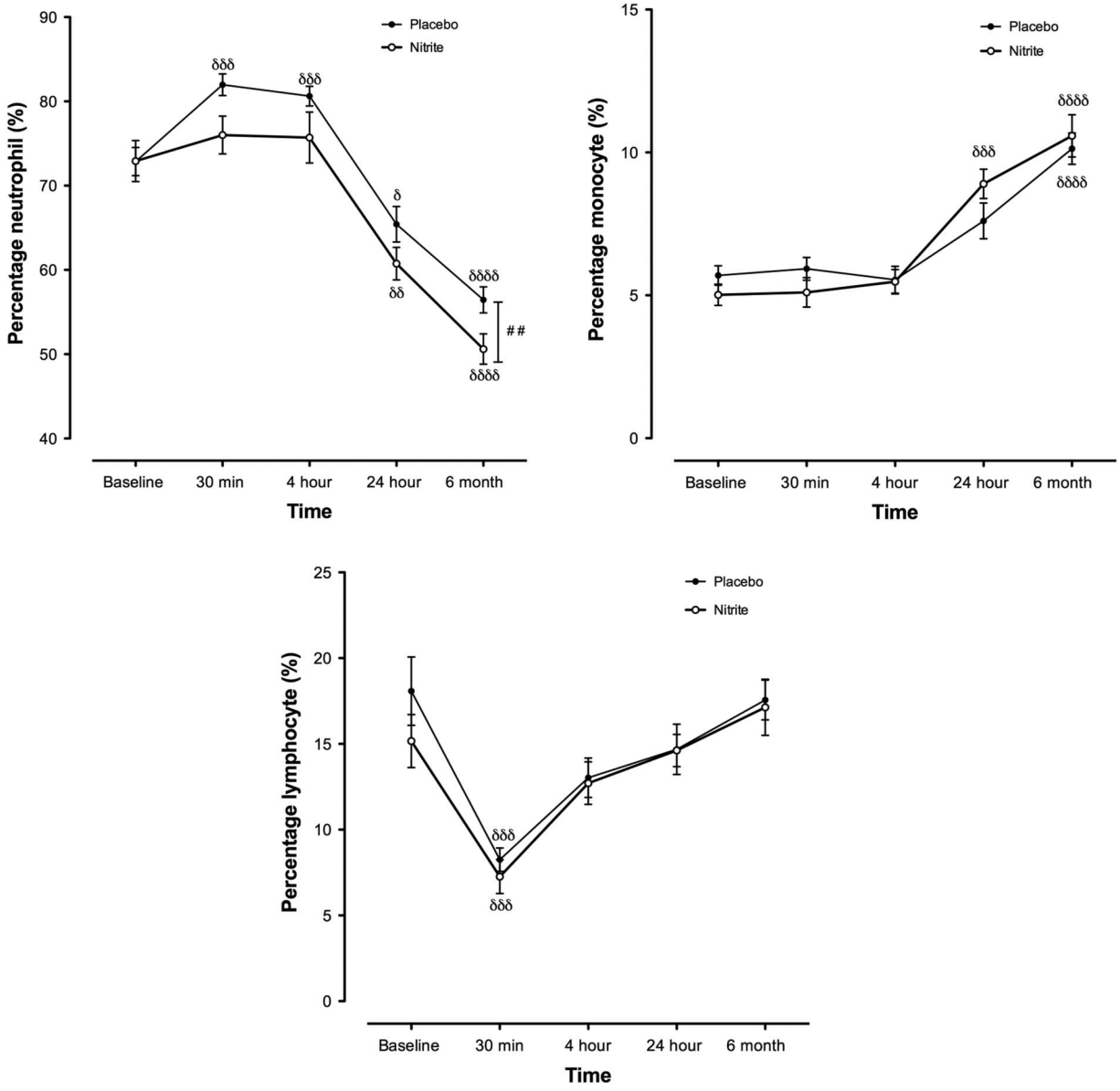

Figure 2 Nitrite treatment suppresses neutrophil numbers following primary percutaneous coronary intervention in ST elevated myocardial infarction (STEMI) patients. Per cent cell subtype present in blood collected from STEMI patients prior to and following primary PCI. Using flow cytometry (A) neutrophils, (B) monocytes and (C) lymphocyte numbers were assessed and determined on the basis of forward and side scatter profiles of 80 patients (40 nitrite and 40 placebo). Data expressed as mean±SEM. For comparisons of change relative to baseline within groups one-way repeated measures analysis of variance (ANOVA) followed by Dunnett's post-test was used with significance shown as δδδ for p<0.001 and δδδδ for p<0.0001. For comparisons between treatments statistical significance is shown as ## for p<0.01 performed using two-way ANOVA.

performed using analysis of variance (ANOVA). Post hoc tests were run only if F achieved $p<0.05$ and there was no significant variance inhomogeneity assessed using GraphPad Prism. Either Bonferroni's post-test for comparisons between groups at specific timepoints or Dunnett's post-test for comparison of responses to baseline measures within treatment groups was conducted. Determination of correlations was performed using either Pearson's correlation coefficient or Spearman's rank correlation coefficient and were expressed as 95% CIs. The cumulative incidence (% of population) of MACE during the follow-up period was estimated by the Kaplan-Meier method; differences were tested using the log-rank test.

RESULTS

Eighty patients were recruited into NITRITE-AMI (40 in the control group and 40 in the nitrite group). All baseline characteristics were similar between the treatment groups (online supplementary table S1). The mean age of the trial participants was 57 years, with 84% male. Twenty-five per cent 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. For full details see Jones *et al*.[4]

Intracoronary nitrite treatment is associated with lower systemic inflammation

Levels of hs-CRP were raised in all patients at baseline and increased over the 24 hours following PPCI; however, the rise in hs-CRP was suppressed in patients receiving nitrite versus placebo (figure 1). At 6 months post-PPCI, circulating levels of hs-CRP were reduced significantly from baseline in the nitrite but not in placebo-treated patients. Correlation analysis of the peak hs-CRP levels at 24 hours with the CK area under the

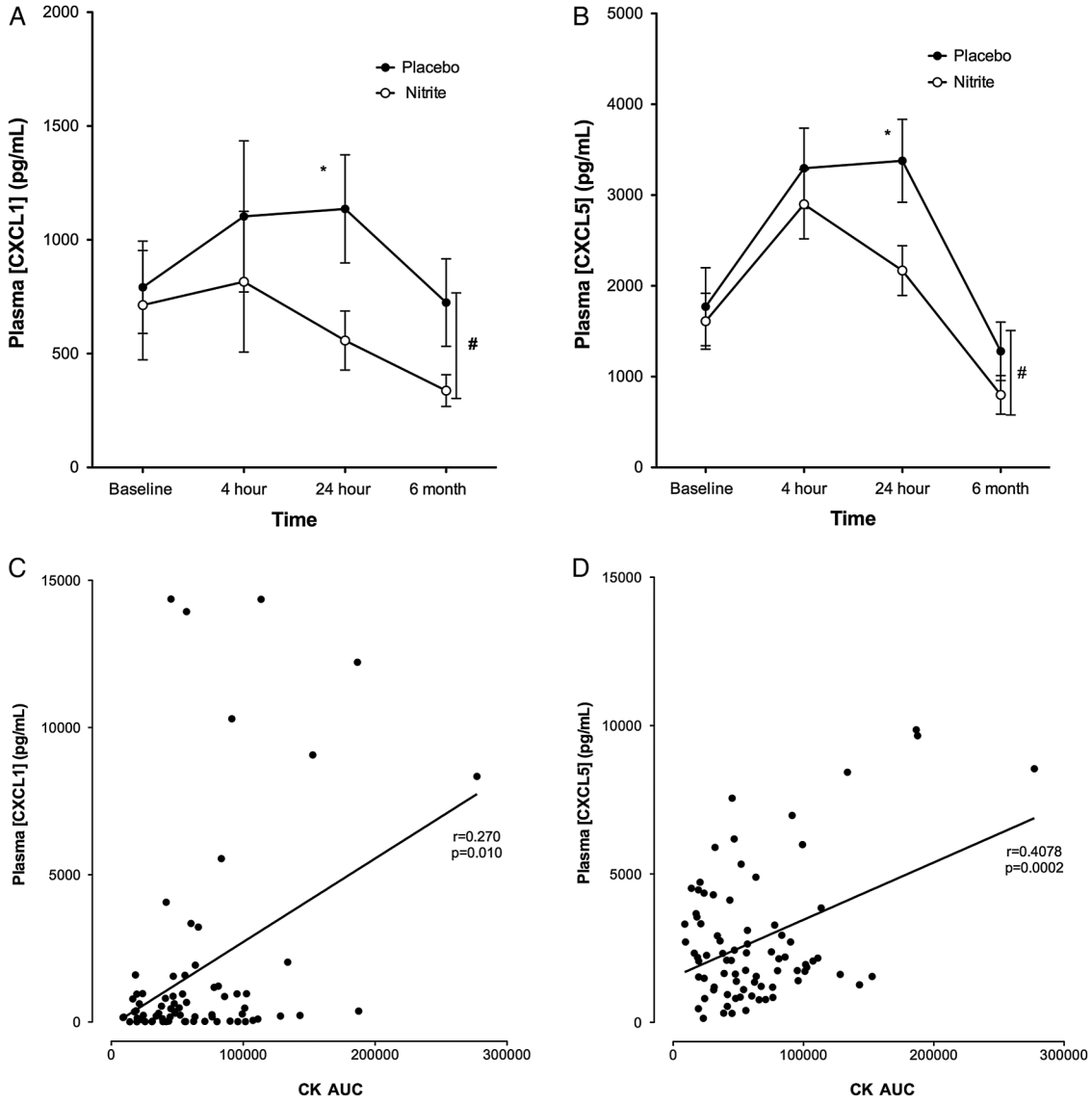

Figure 3 Nitrite-induced changes in plasma CXCL1 and CXCL5 levels. CXCL1 and CXCL5 levels were measured at baseline, 4 hours, 24 hours and 6 months after coronary reperfusion. (A) Shows CXCL1 over time in nitrite-treated versus placebo groups. (B) Shows CXCL5 over time in nitrite-treated versus placebo groups. Data expressed as mean±SEM. Statistical significance determined using two-way ANOVA shown as #=$p<0.05$ with Bonferroni posts-tests to compare timepoints shown as *$p<0.05$. (C) Demonstrates a significant positive association between plasma levels of CXCL1 measured at 24 hours and infarct size assessed by creatine kinase (CK). (D) Depicts a similar positive association between CXCL5 levels at 24 hours and CK area under the curve (AUC). Correlations determined using Pearson's correlation coefficient for linear associations (CD11b) and using Spearman's rank correlation coefficient for non-linear associations (CXCL1). The solid lines represent the least-square fit of the data.

curve (AUC) demonstrated a modest but significant association. Separation of the nitrite group data from the placebo exposed a stronger correlation, although not statistically different from placebo (nitrite: r=0.5574, p=0.0006, placebo: r=0.3829, p=0.0305, p for comparison: 0.14).

Total leucocyte counts were high at baseline remaining elevated at 4 hours but reduced at 24 hours and decreasing further at 6 months (table 1). As with hs-CRP, the total leucocyte count, while identical between the groups at baseline, was significantly attenuated in the nitrite-treated group compared with the placebo group. The differential count identified that lymphocyte numbers between the groups were similar at baseline, 24 hours and 6 months, with lower levels at 4 hours but no evidence of differences between the treatment groups. Monocyte numbers were similar at baseline between the groups remaining unchanged at 4 hours but increasing at 24 hours and declining back to baseline levels by 6 months, with no differences between the treatment groups. In contrast, neutrophil counts varied substantially over the course of 6 months between the groups, with an increase occurring at 30 min post-PPCI, a rise sustained at 4 hours and then progressively decreasing over time (table 1) with a significant reduction in numbers in the nitrite group compared with the placebo group over time (p=0.0079) (table 1), with the greatest difference apparent at 4 hours.

Flow cytometry analysis further supported these observations. Figure 2 demonstrates that immediately post-PPCI there is a rapid (within 30 min) rise in neutrophil number concomitant with a drop in lymphocyte numbers. This is followed by a progressive drop in neutrophil count, recovery of lymphocytes and a rise in monocytes. However, while nitrite treatment did not alter the pattern of monocyte or lymphocyte expression over time, the rise in neutrophil numbers post-PPCI was significantly and selectively suppressed compared with the placebo group.

Nitrite represses neutrophil chemokine expression

Since neutrophil numbers were altered by nitrite treatment, we measured the levels of relevant neutrophil-specific chemokines in plasma. Plasma CXCL1 and CXCL5 levels (chemokines previously implicated in reperfusion-induced neutrophilia) increased at 4 hours from baseline and remained elevated at 24 hours. At 6 months levels had returned to below baseline values (figure 3) in both treatment groups, however, in the nitrite-treated patients the plasma levels of both chemokines postbaseline were substantially reduced compared with placebo (figure 3). Furthermore, post hoc analyses demonstrate a modest positive association between plasma CXCL1 and CXCL5 levels and infarct size (figure 3).

Nitrite treatment is associated with reduced neutrophil CD11b expression

The levels of specific adhesion molecules were measured in a subgroup of patients (23 placebo, 21 in the nitrite group) with similar baseline characteristics to the whole patient cohort (online supplementary table S1). All markers for all cell types were high at 30 min, increased further at 24 hours but dropped to below baseline at 6 months. There were no statistical

Table 2 Levels of activation markers

	Placebo			Nitrite			
Activation marker	30 min	24 hours	6 months	30 min	24 hours	6 months	Significance, p Value
Neutrophil							
CD11b	8.35±0.99	10.10±0.93	4.85±0.40	7.62±0.89	5.79±0.68*	4.30±0.28	0.0034
CD62L	66.57±7.60	83.27±9.27	52.47±4.23	65.35±78.28	78.27±9.45	46.12±4.93	0.4860
CD162	64.89±10.25	99.14±12.19	45.64±7.22	63.85±9.41	89.60±13.66	33.19±5.25	0.3567
CD4 T lymphocyte							
CD11b	5.05±0.87	7.11±1.13	4.33±0.43	4.94±0.66	6.76±0.68	4.18±0.42	0.7449
CD62L	30.13±5.18	34.45±5.48	19.69±3.05	27.96±5.16	36.79±5.67	21.53±3.26	0.8641
CD162	43.08±5.27	68.96±10.99	32.21±3.01	39.43±5.89	69.65±8.79	23.32±3.33	0.4868
CD8 T lymphocyte							
CD11b	4.39±0.47	6.65±0.85	4.73±0.71	4.24±0.54	5.96±0.75	4.28±0.48	0.4352
CD62L	24.69±2.56	27.45±2.60	21.07±2.87	25.18±3.36	28.48±2.47	20.52±2.93	0.8887
CD162	24.95±3.08	52.09±7.82	26.51±2.17	25.93±3.90	51.99±10.85	20.25±2.69	0.7174
Inflammatory monocyte							
CD11b	11.88±2.31	15.72±2.99	7.19±0.91	10.70±0.92	11.56±0.97	5.80±0.82	0.1202
CD62L	69.63±11.64	75.59±9.88	34.88±3.80	71.11±9.51	77.27±11.53	31.20±4.05	0.9810
CD162	95.59±23.13	168.79±25.72	42.09±4.67	94.40±15.89	128.23±14.65	36.81±6.16	0.2744
Resident monocyte							
CD11b	7.05±0.91	9.56±1.22	4.27±0.41	7.52±1.01	6.73±0.91	3.76±0.34	0.1796
CD62L	42.63±6.17	53.68±10.77	18.80±1.88	37.34±4.46	36.93±6.94	18.31±2.29	0.1442
CD162	35.29±7.37	76.33±14.75	23.29±2.04	36.50±6.30	60.03±11.51	21.01±2.92	0.4291
Intermediate monocyte							
CD11b	11.56±2.21	19.77±6.06	6.68±1.22	10.67±1.29	12.65±1.41	5.19±0.88	0.1904
CD62L	12.96±1.65	17.89±2.52	7.54±0.81	12.51±1.44	14.74±1.76	7.21±0.66	0.3203
CD162	29.41±6.55	72.23±16.48	21.15±3.34	39.19±7.26	66.41±13.68	14.77±3.73	0.9200

Blood samples for activation marker expression were collected at 30 min, 24 hours and 6 months post-PCI in 44 patients (23 nitrite, 21 placebo). Data expressed as mean±SEM. Statistical analysis conducted using repeated measures two-way ANOVA with p values indicated in the table. Bonferroni's post-test was conducted comparing specific timepoints between the nitrite and placebo groups and is shown as *p<0.05.
ANOVA, analysis of variance; PCI, percutaneous coronary intervention.

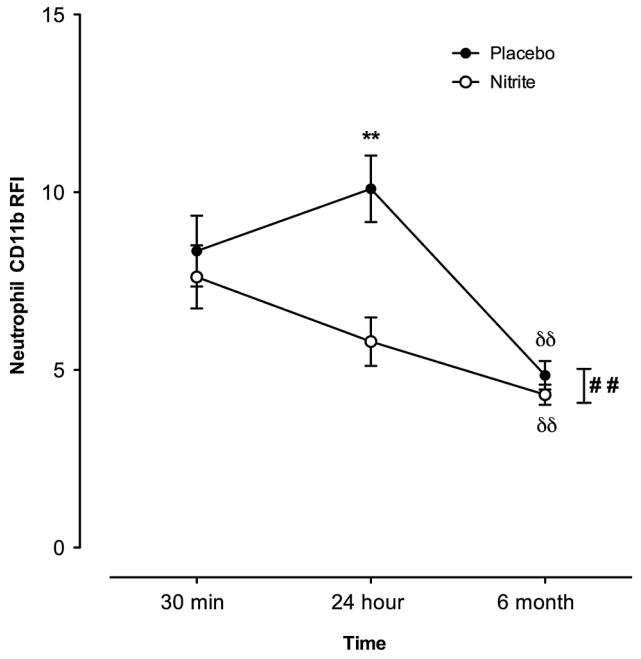

Figure 4 Nitrite treatment suppresses neutrophil CD11b in ST elevated myocardial infarction patients. Relative fluorescence intensity (RFI) for CD11b was measured at 30 min, 24 hours and 6 months after coronary reperfusion. RFI was assessed in 44 patients (23 placebo patients and 21 nitrite patients). Data expressed as mean±SEM. For comparisons of change relative to baseline within groups, one-way repeated measures analysis of variance (ANOVA) followed by Dunnett's post-test compared with the 30 min timepoint was used with significance shown as δδ for p<0.01. For comparisons between treatments statistical significance is shown as ## for p<0.01 performed using two-way ANOVA and **p<0.01 for Bonferroni's post-test comparing specific timepoints between groups.

differences in the temporal changes in expression of any of these activation markers between the treatment groups except for CD11b expression on neutrophils (table 2), which was significantly and selectively reduced in the nitrite-treated patients compared with the placebo-treated patients (figure 4).

Association of neutrophil numbers and activation state with infarct size

Post hoc correlation analyses demonstrate that infarct size correlates directly with neutrophil but not monocyte or lymphocyte numbers (online supplementary figure S2). This correlation was stronger in the nitrite versus the placebo group (nitrite: r=0.5878, p≤0.0001, placebo: r=0.4156, p=0.008, p for comparison: 0.066). In addition, CD11b and CXCL1 expression are also modestly correlated to CMR-determined infarct size and inversely to myocardial salvage index (figure 5).

Nitrite reduces MACE at 3 years postintervention

Figure 6 depicts Kaplan-Meier curves for MACE and demonstrates sustained long-term reductions in MACE in the nitrite group compared with the placebo group.

DISCUSSION

The magnitude of the inflammatory response triggered by reperfusion is strongly associated with outcome in patients with STEMI treated with PPCI. Accordingly, approaches that reduce this inflammatory response might prove useful in improving outcomes following PPCI. In this exploratory, mechanistic study, we demonstrate that intracoronary treatment with nitrite in patients undergoing PPCI is associated with a reduction in the inflammatory response. We show a selective repression by nitrite of reperfusion-induced neutrophilia, a phenomenon known to be associated with worsening outcome, in addition to reducing cell activation reflected by reduced expression of the adhesion molecule CD11b. In addition, reduced levels of the neutrophil chemoattractant chemokine, CXCL1, and the neutrophil-derived monocyte chemoattractant chemokine, CCL2, were associated with nitrite treatment. Together, these exploratory data suggest that at least a component of the beneficial effects of intracoronary nitrite administration prior to reperfusion in patients with STEMI undergoing PPCI likely relates to suppression of the pro-inflammatory neutrophil response to acute reperfusion.

AMI evokes an intense inflammatory response with release of pro-inflammatory cytokines including tumour necrosis factor-α, interleukin-6, CXCL1 and CXCL5 within 24 hours after reperfusion[22 23]. Patients with large infarcts often have signs compatible with a systemic inflammatory response syndrome reaction[24 25] with the scale of the response reflecting infarct size, and excessive early inflammation associated with adverse left ventricular remodelling and poor outcomes.[26 27] In this study, the elevated levels of hs-CRP (normal is <2 pg/mL) and total circulating leucocytes at baseline reflect this systemic inflammatory response. In addition, the rise in hs-CRP postreperfusion confirms a second hit reperfusion-induced inflammatory response.[27 28] In the nitrite-treated patients, this rise in hs-CRP levels was suppressed suggesting a reduced inflammatory response in this group. It is unlikely that the reduced levels of hs-CRP reflect a direct action of nitrite to result in reduction of hepatic-CRP release since circulating levels of nitrite, while raised, did not rise to levels that would be expected to induce such an effect. Since stimulation of CRP is thought to be a downstream event triggered by cytokines and chemokines induced by leucocytes activated within the myocardium, we suggest that nitrite likely targeted the local inflammatory response.

Higher levels of white blood cells (WBC), and specifically neutrophils, post-PPCI have been associated with increased infarct size and impaired left ventricle function,[19 29] with some data suggesting that WBC count postprocedure better predicts outcome than baseline levels.[29] This rise in WBC and neutrophil count after AMI takes several hours to subside, with the apparent rate of decrease reflecting the success of reperfusion therapy and correlating with TIMI flow and myocardial blush grade (MBG).[16] Our data are consistent with this, demonstrating a decline in WBC count early (4 hours) after reperfusion that was greater in the nitrite-treated patients and associated with improved ST segment resolution (a similar measure to MBG) and smaller infarct size. Importantly, while changes in all cell subgroups occurred over time, nitrite treatment only altered the neutrophil profile, suggesting a selective action of nitrite for this cell type. Previous studies in preclinical models support this observation with anti-inflammatory and antineutrophil effects of endogenously generated NO during myocardial IR injury.[30 31]

The early recruitment of neutrophils in myocardial IR injury[22] is driven by the expression and release of neutrophil chemokines which includes CXCL5 and CXCL1.[23] While the effects of direct targeting of these individual chemokines has not been reported, smaller infarcts following myocardial IR were evident in mice lacking CXCR2,[32] the key receptor for the CXCL chemokines, or following a single administration of the

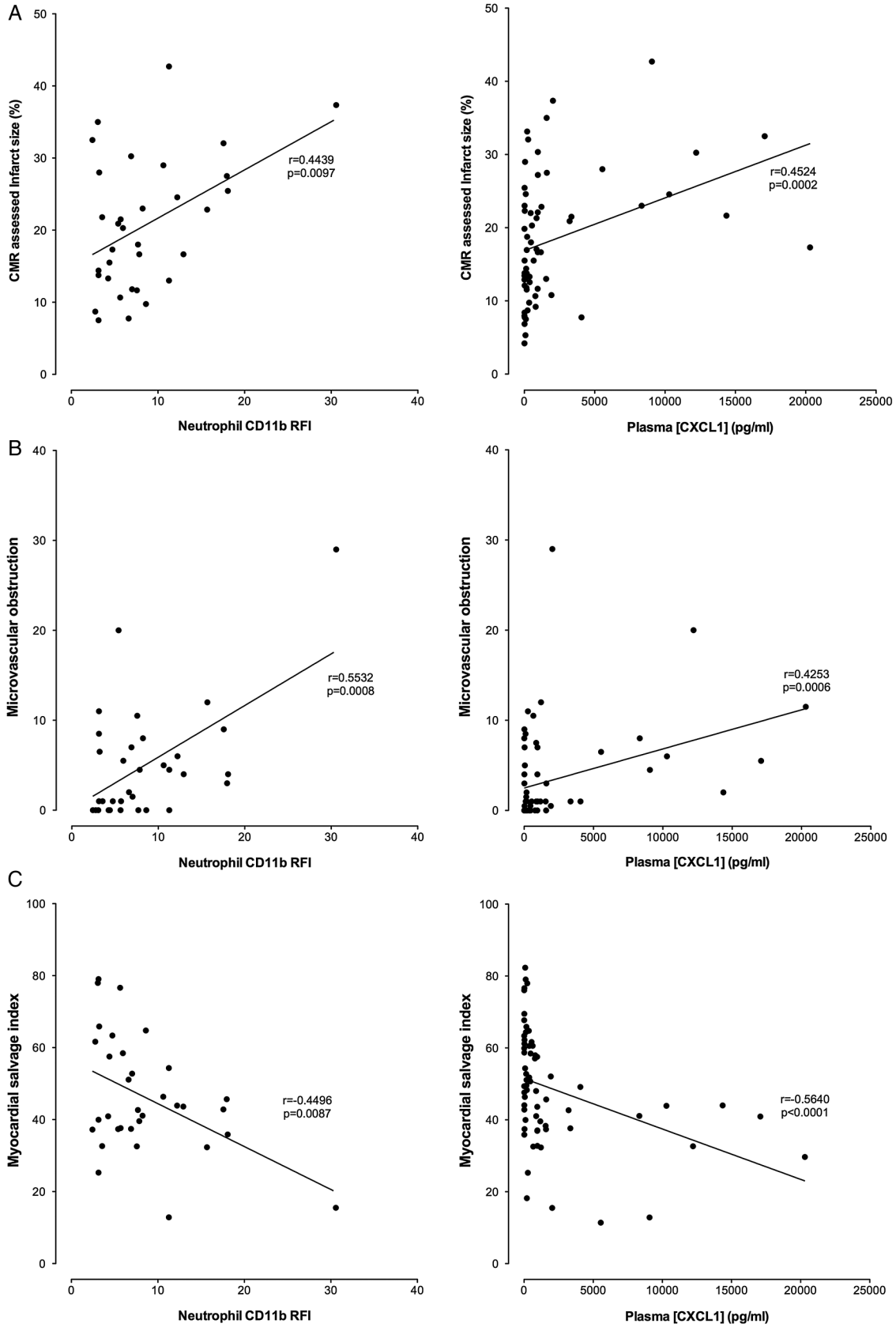

Figure 5 Associations between neutrophil CD11b/CXCL1 and cardiac magnetic resonance imaging (CMR) measurements. There was a significant positive association between neutrophil CD11b and CXCL1 levels counts at 24 hours and infarct size assessed by CMR as shown in panel A. Panel B depicts a similar positive association between neutrophil CD11b and CXCL1 levels counts at 24 hours and infarct size assessed by CMR. Panels C and D show a negative correlation between neutrophil CD11b and CXCL1 levels counts at 24 hours and the myocardial salvage index. Correlations determined using Pearson's correlation coefficient for linear associations (CD11b) and using Spearman's rank correlation coefficient for non-linear associations (CXCL1). The solid lines represent the least-square fit of the data.

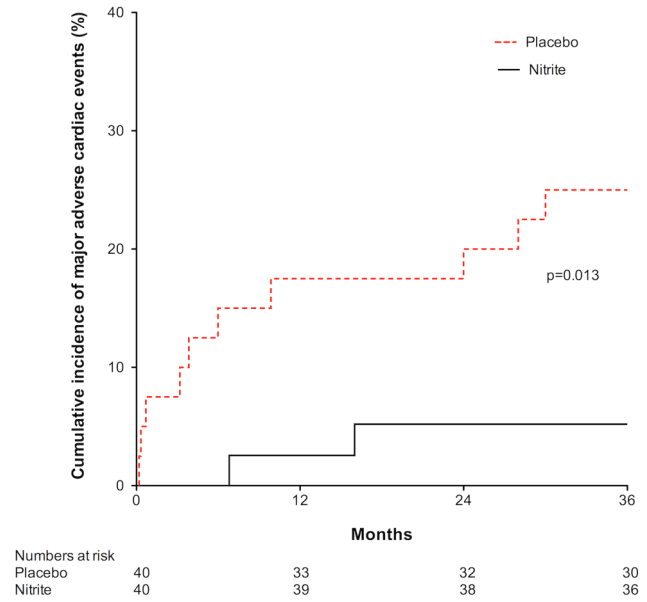

Figure 6 Reduced major adverse cardiac events (MACE) at 3 years postnitrite treatment. The cumulative incidence (% of population) of MACE during the 3-year follow-up period was estimated by the Kaplan-Meier method; differences were tested using the log-rank test.

CXC chemokine-binding protein, Evasin-3, which prevented CXCL1-induced neutrophil recruitment and reactive oxygen species production in the myocardium.[33] Therefore, the reduced levels of CXCL1 and CXCL5 seen in the nitrite-treated patients likely resulted in reduced neutrophil activation, and thus reduction in infarct size. Neutrophil recruitment is triggered by the increased expression of key adhesion molecules on the cell critical for neutrophil rolling (PSGL-1 and CD62L), adherence and tethering (CD11b), all of which were raised by AMI. Nitrite treatment selectively reduced the expression of CD11b on neutrophils only; having no effect on all other markers on all cell types assessed, suggesting a selectivity of nitrite for neutrophils rather than a direct effect on CD11b expression.

Study limitations

NITRITE-AMI was powered off a reduction in myocardial infarct size rather than the systemic inflammatory response meaning the study may lack power. This is especially true of the inflammatory marker expression (CD11b, CD62L and CD162) on systemic leucocytes as this was only measured in 46 out of the 80 patients. The data in this study are generated with a strong signal suggestive of a nitrite effect. Further prospective studies powered to measure differences in inflammatory markers to confirm these observations are now warranted.

CONCLUSION

Important reductions in the systemic inflammatory response post-PPCI were associated with nitrite treatment. Specifically, in nitrite-treated patients a sustained long-term reduction in MACE in the nitrite group compared with the placebo group was associated with a selective repression of neutrophil activation. These results suggest that the apparent beneficial effects of nitrite in patients with STEMI may be due to a specific localised suppression of neutrophil activation during reperfusion. Further large-scale studies powered to assess this prospectively are warranted.

Key messages

What is already known on this subject?
Inflammation is a critical contributor to inadequate reperfusion, myocardial damage and death postprimary percutaneous coronary intervention (PPCI) for acute myocardial infarction. Thus, strategies targeting this inflammatory response may offer opportunities for improving outcomes. We investigated whether intracoronary nitrite might exert anti-inflammatory effects in the setting of PPCI.

What might this study add?
A single dose of nitrite at PPCI is associated with a reduction in the circulating inflammatory response, specifically due to reduced neutrophil numbers and activation state; an effect associated with reduced major adverse cardiac events at 3 years.

How might this impact on clinical practice?
The sustained long-term beneficial effect of a single bolus intracoronary nitrite administration potentially offers a simple and easy-to-administer adjunctive therapy that further improves outcomes following PPCI.

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IMAGE CHALLENGE

A case of recent myocardial infarction with cardiac failure

For the question see page 499

ANSWER: C

The echocardiogram illustrates an akinetic LAD territory, severe LV dysfunction and mild pericardial effusion. The LV apex shows alternating hyperechoic and hypoechoic areas with no colour turbulence demonstrable within the echolucent region. An absence of a narrow communication with ventricular cavity or interruption of LV wall continuity rules out contained LV rupture. A wide neck with a ratio of the maximum diameter of the orifice to the maximum internal diameter of the aneurysmal cavity >0.5 and lack of a turbulent Doppler flow at aneurysm neck in a patient with recent anterior wall myocardial infarction go more in favour of a true ventricular aneurysm.[1] The stagnation of blood, contact with procoagulant fibrous tissues in the aneurysmal cavity and hypercoagulable state of an acute coronary syndrome result in mural thrombus formation.[2] Autolysis of an organised apical thrombus which is evident in the zoomed apical view of echocardiogram gives this appearance of alternating hyperechoic and echolucent areas. A transaneurysmal approach during the endoventricular circular patch plasty (Dor procedure) revealed an intact ventricular wall with chunks of layered thrombus in the cavity (see online supplementary figure S1).

Intramyocardial dissecting haematoma progresses along natural planes between the ventricular spiral muscles resulting in avulsion of perforating vessels. Thus an echolucent blood filled cavity is lined on outer side by myocardium and pericardium while inner wall is composed of myocardium and endocardium.[3] However, multiple alternating layers of echodense and echolucent spaces with absence of a dissection flap makes this diagnosis unlikely. The absence of echocardiographic features of non-compaction during initial admission and lack of blood flow into the suspected intertrabecular recesses rule out option D.[4]

Distinction between these entities is often perplexing but forms the cornerstone of a successful case management. In doubtful cases, cardiac MRI helps in better tissue characterisation.

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Heart

Intracoronary nitrite suppresses the inflammatory response following primary percutaneous coronary intervention

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