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The safety and efficacy of intracoronary nitrite infusion during acute myocardial infarction (NITRITE-AMI): Study protocol of a randomised controlled trial.

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The safety and efficacy of intracoronary nitrite infusion during acute myocardial infarction (NITRITE-AMI): Study protocol of a randomised controlled trial.

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Abstract

Introduction

Acute myocardial infarction (AMI) is a major cause of death and disability in the UK and worldwide. Presently, timely and effective reperfusion with primary percutaneous coronary intervention (PPCI) remains the most effective treatment strategy for limiting infarct size, preserving left ventricular ejection fraction (LVEF), and improving clinical outcomes. However, the process of reperfusion can itself induce cardiomyocyte death, known as myocardial reperfusion injury, for which there is currently no effective therapy. Extensive pre-clinical evidence exists to suggest that sodium nitrite (as a source of endogenous nitric oxide) is an effective therapeutic strategy for preventing myocardial reperfusion injury. The purpose of NITRITE-AMI is to test whether sodium nitrite reduces reperfusion injury and subsequent infarct size in patients undergoing PPCI for MI.

Methods and design

NITRITE-AMI is a double-blind, randomized, single-centre, placebo-controlled trial to determine whether intra-coronary nitrite injection reduces infarct size in patients with myocardial infarction undergoing primary angioplasty. The study will enrol 80 patients presenting with ST-elevation myocardial infarction. Patients will be randomised to receive either a bolus of intra-coronary sodium nitrite or placebo (sodium chloride) at time of PPCI. The primary outcome is

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Infarct size assessed by Creatine Kinase area under the curve (AUC) over 48 hours. Secondary endpoints include Troponin T AUC and infarct size, LV dimensions and myocardial salvage index assessed by CMR, markers of platelet reactivity and inflammation, the safety and tolerability of intra-coronary nitrite, and 1 year major adverse cardiac events.

Ethics and dissemination

The study is approved by the local ethics committee (NRES Committee London West London: 11/LO/1500) and by the Medicines and healthcare products Regulatory Agency (MHRA) (EudraCT nr. 2010-022460-12). The results of the trial will be published according to the CONSORT statement and will be presented at conferences and reported in peer-reviewed journals.

Trial registration: United Kingdom Clinical Research Network (Study ID 12117), http://clinicaltrials.gov (NCT01584453) and Current Controlled Trials (ISRCTN:38736987).

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Article focus

This article describes the study protocol of a 1st in man study assessing the safety and efficacy of intra-coronary nitrite infusion during primary percutaneous coronary intervention (PPCI) for acute myocardial infarction (AMI) in a randomized double-blind placebo control trial.

Key messages

Despite the introduction of PPCI for treatment of AMI significant morbidity and mortality rates remain, mainly due to reperfusion injury contributing to infarct size.

Reducing reperfusion injury is a major target in improving these outcomes.

There is extensive pre-clinical data demonstrating efficacy of sodium nitrite (delivered locally) in reducing reperfusion injury and subsequent infarct size.

Strengths and limitations of the study

This is the first randomised controlled trial assessing the use of intra-coronary nitrite to reduce infarct size during PPCI for STEMI.

It is a single centre study, which can affect applicability of the results to other units.

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Introduction

Coronary heart disease is the commonest cause of death in the UK, in the main as a consequence of acute myocardial infarction (AMI)) causing 1 in 5 and 1 in 7 deaths in men and women respectively (www.heartstats.org). Presently, timely and effective reperfusion with primary percutaneous coronary intervention (PPCI) for ST-elevation myocardial infarction (STEMI) remains the most effective treatment strategy for reducing myocardial infarct (MI) size, preserving left ventricular (LV) ejection fraction, and preventing the onset of heart failure[1 2 3 4]. However, despite the introduction of PPCI, and other advances such as the introduction of anti-platelet therapies, resulting in a reduction in mortality of ~50% at 30 days, substantial mortality and morbidity rates still persist[5 6]. The mortality of STEMI patients at 30 days undergoing PPCI in Europe remains significant (3–7% in-hospital mortality depending on the country)[7] and 15-20% of patients progress to develop heart failure[8]. One of the main determinants of prognosis after AMI is the size of the infarct[9 10 11], and importantly increased infarct size is associated with an increased incidence of heart failure and arrhythmias[12 13 14]. Thus, there is a clear need for identification of additional strategies that might decrease infarct size and improve outcome.

Reperfusion injury and infarct size

In the setting of STEMI the immediate reopening of acutely occluded coronary arteries via PPCI is the treatment of choice to salvage the ischaemic

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myocardium. However, the sudden re-initiation of blood flow leads to a local acute inflammatory response with further endothelial and myocardial damage. This phenomenon, described as 'reperfusion injury'[15] may explain why, despite optimum myocardial reperfusion, the short-term mortality after AMI approaches 7% [6] and the incidence of heart failure approaches 15-20%[1,14]. Experimental in vivo models suggest that whilst 50% of the final infarct size is due to the ischaemic insult that the remaining 50% is due to reperfusion injury [16]. Although, the process of myocardial reperfusion continues to be optimized with recent advances in PPCI technology (thrombus aspiration, novel stents) [17], anti-platelet (Prasugrel, Ticagrelor) [18] and anti-thrombotic therapy (Bivalirudin)[19], there is currently no effective therapy for reducing myocardial ischaemia-reperfusion (I/R) injury per se.

Reducing reperfusion Injury

Disappointingly, whilst several strategies are effective in reducing I/R injury in pre-clinical models, the majority of these approaches have not translated to the clinical setting with the notable exception of cyclosporine [20]. However, more recently a potential solution to the problem of reperfusion injury has been proposed in the form of inorganic nitrite (NO_2^-). Over the last decade evidence has accumulated supporting the view that NO_2^-, which is abundant in blood and tissues[21 22 23 24], represents a significant stable intravascular endocrine reservoir and tissue storage form of nitric oxide (NO) that exerts a number of beneficial effects [25].

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Inorganic nitrite

Under normal conditions, basal endogenously generated NO, produced via the conventional L-arginine/NO synthase (NOS) pathway is essential for maintaining homeostasis, in particular, by sustaining normal healthy cardiac function, perfusion and cardioprotection[26 27]. However, during ischaemia the activity of the predominant isoform that underlies this NO synthesis, i.e. endothelial NOS (eNOS), is severely attenuated as a result of inadequate delivery of oxygen and co-factors[28]. In addition, reperfusion generates oxidative stress, which also further reduces the bioavailability of NO[29 30], thereby removing its cardioprotective effects. The cardioprotective effects of NO have been proposed to relate to a number of actions that combine to provide an overall beneficial profile. Firstly, NO exerts potent vasodilator effects in the ischaemic myocardium[31], which allows for essential perfusion of injured tissue. In addition NO exerts anti-inflammatory effects inhibiting leukocyte recruitment[32 33] and thus suppressing the inflammatory cell-driven injury to the endothelium and perivascular myocardium that plays a major role in determining infarct size post myocardial I/R injury[34]. NO also exerts anti-platelet effects, which together with the anti-leukocyte actions of NO attenuates capillary plugging[35]. Finally, deficient NO generation contributes to the delay in re-endothelialisation following PPCI, enhances cardiomyocyte death and apoptosis during I/R injury, and in the long term contributes to cardiomyocyte hypertrophy post-infarct and decreases the impact of strategies that might facilitate new cardiomyocyte generation[36 37]. Together, such pre-clinical observations are consistent with a protective role for NO [35]

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and support the concept of pharmacotherapy focused on replacing the protective NO 'lost' during myocardial I/R injury.

Pre-clinical evidence for beneficial effects of nitrite

NO_2^- has now been shown to mediate a number of beneficial effects in the cardiovascular system. Perhaps the most potent and reproducible effect of NO_2^- being its ability to mediate cytoprotection after I/R in a number of organs and species[38 39 40 41 42]. The activity of nitrite resides in its propensity for conversion to NO: the optimal conditions for which are low $p0_2$ and low pH, conditions which prevail during ischaemic episodes and coincide with substantial reduction of NOS function[28]. Studies from our own lab[38] and those of others have demonstrated nitrite-derived NO production in the heart and blood vessels[22 23 24].

The beneficial effects of inorganic nitrite in the heart were first demonstrated in 2004[38] where administration of NO_2^- either prior to or at reperfusion in the isolated rat Langendorff heart preparation improved both left ventricular function and coronary perfusion pressure as well as decreasing infarct size following an I/R insult. Several studies followed confirming these beneficial effects in the heart demonstrating the protective effects of nitrite administered intra-ventricular or intra-coronary in rodent models of AMI in vitro and vivo[38 39 43 44]. Importantly, studies have confirmed that ischaemia-dependent conversion of NO_2^- to protective NO is also a property shared by human heart tissue ex-vivo[38]. The functional benefits of NO_2^- have been translated to larger species in vivo, where the intravenous administration of NO_2^- in just the last 5

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minutes of a 2 hour period of ischaemia in the heart, reduced infarct size by ~50% compared in dogs[45]. Although, the largest body of evidence demonstrating the cytoprotective properties of NO_2^- has accumulated in models of MI, similar protective effects have been evidenced in other organs subject to I/R injury including the liver[39], brain[40] and kidney[42]. In all of these studies the beneficial effects were shown to be due to the activity of NO and were specifically associated with the local application into or on the organ of interest.

These observations clearly support investigation of the potential of NO_2^- in the treatment of acute STEMI, particularly where NO_2^- could be delivered locally before balloon inflation at the time of PPCI. The design of this study seeks to determine whether a significant improvement in infarct size and clinical outcomes can be achieved by the intra-coronary injection of nitrite during PPCI.

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Methods and analysis

Study Design

NITRITE-AMI is a double-blind, randomized, single-centre, placebo-controlled trial to determine whether nitrite injection reduces infarct size in patients with myocardial infarction undergoing primary angioplasty (Figure 1).

Aims

i) To assess whether intra-coronary nitrite is safe, tolerable and reduces infarct size during PPCI in patients with AMI

ii) To understand the effect of intra-coronary nitrite on inflammatory load and platelet function during PPCI for AMI.

Hypothesis: In patients with STEMI undergoing PPCI, an intra-coronary injection of nitrite initiated prior to establishment of full reperfusion reduces infarct size through prevention of I/R injury.

Study participants

This is a single-centre trial with 80 patients to be recruited at The Barts Health Heart Attack Centre, based at The London Chest Hospital. This centre runs a 24/7 Heart Attack Service covering a population of almost two million people from the City of London to the M25 and performed 755 primary angioplasties

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in 2012. Haemodynamically stable patients aged between 18 and 80, presenting with STEMI, with a single culprit vessel will be recruited. Potentially eligible patients will be approached at their acute admission (Table 1 for inclusion criteria and table 2 for exclusion criteria).

Randomisation and study blinding

Patients will be randomised to receive sodium nitrite or matching placebo (in a 1:1 allocation) with both the patient and treating clinicians blinded to the assignment (double-blind). Manufacture of the Investigational medicinal product, blinding, coding and randomisation will be carried out by the pharmacy manufacturing unit at Ipswich hospital prior to transfer of stock to the London Chest Hospital pharmacy. The randomisation list will be generated by a computer-generated randomization table (http://www.randomization.com) based on blocks of ten to assign patients to treatment group or placebo group. 80 indistinguishable vials of sodium nitrite and placebo will be provided and delivered to patients in a sequential fashion. Only the pharmacy at the London Chest Hospital and the manufacturing unit in Ipswich will be aware of the identity of the solution.

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Table 1. Inclusion Criteria

- Patients aged between 18 and 80 years
- Acute STEMI with ECG showing ST-segment elevation of 1mm or more in two adjacent limb leads or 2mm or more in at least two contiguous precordial leads or new left bundle branch block;
- Haemodynamically stable
- Estimated symptom to balloon or aspiration time < 6 hours
- Angiographicallly
    - PPCI indicated for revascularisation
    - Single epicardial artery to be treated
    - Expected ability to use over the wire balloon

Table 2. Exclusion Criteria

- Inability to consent (including inability to speak English)
- Patients on organic nitrate treatment (Nicorandil, isosorbide mononitrate)
- Previous history of AMI, systolic dysfunction or CABG
- Subjects presenting with cardiogenic shock (systolic blood pressure <80 mmHg for >30 minutes, or requiring inotropes or emergency intra aortic balloon pump for hypotension treatment) or cardiopulmonary resuscitation
- Current diagnosis of or treatment for malignancy, other than non-melanoma skin cancer.
- Current life-threatening condition other than vascular disease that may prevent a subject completing the study.
- Use of an investigational device or investigational drug within 30 days or 5 half-lives (whichever is the longer) preceding the first dose of study medication.
- Patients considered unsuitable to participate by the research team (e.g., due to medical reasons, laboratory abnormalities, or subject's unwillingness to comply with all study-related procedures).
- Severe acute infection, or significant trauma (burns, fractures).
- Pregnancy.
- Contra-indications to CMR scanning
    - Pacemakers, intracranial clips or other metal implants
    - claustrophobia
    - renal failure (eGFR<30mls/min)
- History of alcohol or drug abuse within the past 6 months
- History of congenital methaemoglobinaemia.
- Angiographically
    - Severe vessel tortuosity, diffuse disease or severe calcification is present which may impede successful delivery of the over the wire balloon

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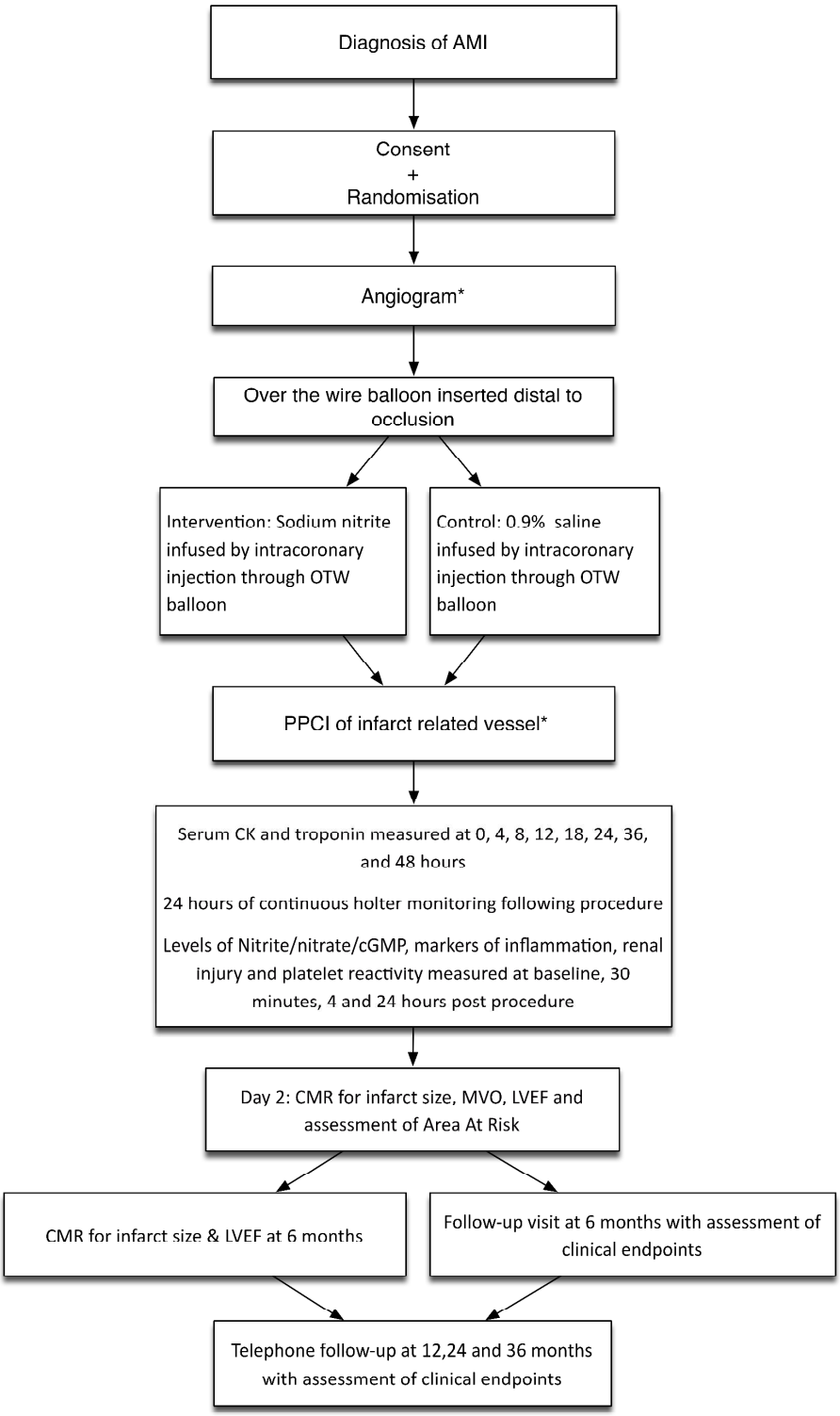
Figure 1. Flow diagram of Study design



AMI: acute myocardial infarction, OTW: Over the wire balloon, PPCI: primary percutaneous coronary intervention, CMR: cardiac magnetic resonance imaging, MVO: microvascular obstruction, LVEF: Left ventricular ejection fraction

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Intervention

The experimental intervention is a bolus of sodium nitrite solution, 1.8 micromol in 10 ml (pre-diluted in 0.9% sodium chloride in a syringe) which will be delivered over 30 seconds via intracoronary injection initiated during the re-establishment of antegrade epicardial flow with PPCI. The control intervention is a bolus of 0.9% sodium chloride solution (prepared with an identical appearance to the sodium nitrite). The patient, the PPCI operator, and the assessor of clinical outcomes will be blinded to the treatment allocation

After crossing the obstruction of the infarct-related coronary artery with a long guide wire, an over-the-wire balloon will be positioned at the level of the obstruction. The guide wire will then be removed and the sodium nitrite or placebo will be injected by hand through the central lumen of the balloon catheter into the distal vascular bed over 30 seconds. The guide wire will then be reinserted through the balloon catheter and advanced to a distal position. The procedure will then be continued as per standard practice. The intracoronary route allows for the nitrite to be delivered in an adequately high and effective local concentration with negligible effects on systemic levels (due to its uptake locally into ischaemic myocardium, short half-life of ~1 min and systemic dilution). The dose of 1.8 micromol is derived from clinical studies in human forearms where this dose resulted in a local concentration between 2.5 and 10 micromol/l, well within the range associated with protection in reperfusion injury[46 47 48].

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Study Endpoints

Blood samples for Troponin-T and Creatine Kinase (CK) will be taken prior to PPCI and at 4, 8, 12, 18, 24, 36 and 48 hours post-procedure. Blood sample for full blood count (FBC), urea and electrolytes (U+E's), liver function tests (LFT's), HbA1c, glucose, total cholesterol, will be taken at baseline. Arterial blood gases will be taken pre and post PPCI to assess levels of methaemoglobin. NT-pro BNP will be measured at baseline and 48 hours. Blood will be collected for flow cytometry assessment of inflammation and platelet reactivity at baseline, 30 minutes, 4 and 24 hours post PPCI. Whole blood aggregometry will be used for assessment of platelet reactivity. Plasma nitrite, nitrate and cyclic guanosine monophosphatase (cGMP) (surrogate for NO) levels will be measured at baseline, post procedure and at 4, and 24 hours post PPCI. Cardiac MR (CMR) will be performed 2 days and 6 months after the index procedure.

The primary endpoint is myocardial infarct size, assessed by 48 hour area under the curve (AUC) plasma creatine kinase (CK) levels. Creatine Kinase AUC is a recognized accurate estimate of infarct size[49 50] used in multiple previous studies assessing both thrombolytic[51] and mechanical reperfusion[20]. Secondary endpoints include alternative measures of myocardial infarct size (48 hour AUC Troponin T, and CMR-determined infarct size, myocardial salvage index, LV volumes, mass and ejection fraction, and the presence of microvascular obstruction (MVO)), and mechanistic endpoints (plasma nitrite/cGMP) concentrations/and markers of inflammation/platelet reactivity

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measured at baseline, post procedure, at 4 hours and 24 hours post-PPCI). Clinical endpoints include the acute safety and tolerability of intra-coronary nitrite in STEMI (haemodyamics and in-patient major adverse cardiac events (MACE)), assessment of ventricular rhythm disturbance for 24 hours post PPCI and an assessment of MACE endpoints at 6 and 12 months (death, heart failure, MI, stroke, need for repeat revascularisation).

CMR imaging and analysis

CMR imaging will be performed 2 days and 6 months ± 2 weeks following study drug administration using a 1.5-T scanner (Philips Medical Systems, Best, The Netherland). Each examination will use cine-CMR for ventricular volumes and function, and DE-CMR for infarct size assessment and evaluation of MVO. Myocardial oedema will be assessed at all time points using T2-weighted triple inversion turbo spin echo STIR imaging (TE 80, TR 1667). The inversion time will be optimised to null normal myocardium. Images will be anonymised, batched and analyzed in a blinded fashion by 2 experienced operators. Scar and oedema volumes will be calculated by manually drawing endocardial and epicardial contours followed by semi-automated selection of normal remote myocardium per slice. Myocardial oedema will be described as >2SD in signal intensity from remote normal myocardium. Infarct size will be calculated using the full-width half maximum method as previously described [52].

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Myocardial salvage index

When assessing the efficacy of a reperfusion treatment strategy, it is essential to express myocardial infarct size (IS) as a percentage of the area-at-risk (AAR). In this study, the AAR will be quantified using both coronary angiography (modified Bypass Angioplasty Revascularisation Investigation [BARI][53] and modified Alberta Provincial Project for Outcome Assessment in Coronary Heart Disease [APPROACH][54] jeopardy scores) and the acute (2 day) CMR scan (infarct endocardial-surface-area [Infarct-ESA]). As a measure of the AAR, infarct-ESA has been validated against the BARI and modified APPROACH scores[54] and T2-weighted imaging of myocardial oedema[55]. Myocardial salvage index will be calculated according to (AAR-IS)/AAR.

Adverse events (AE) reporting

Safety analyses will summarise all AEs, serious AEs and related unexpected serious AEs. The number of events and number of patients with events will be summarised. All serious adverse events (SAEs) that are thought to be related to treatment are subject to expedited reporting and will be reported within 24 hours of study team becoming aware. The principal investigator will be responsible for follow-up of all SAEs to ensure all details are available and for reporting to the regulatory authorities.

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Statistical Analysis

To calculate the target sample size for the trial, we used the available database of the study of myocardial postconditioning by Staat et al[49] and a recent study assessing cyclosporine[11] for infarct size reduction. We hypothesized that nitrite would reduce the AUC for creatine kinase release by 30%, the same amount as both cyclosporine and postconditioning. For a statistical power of 80% and a probability of a type I error of 0.05 using a two-sided test, we calculated that the sample size should be 70 subjects (35 per group). Since 4-8% of patients will die by the time of the primary endpoint at 6 months and 10% will either not tolerate or fail to attend the MRI at 6 months an additional 10 patients will be needed, giving a total of 80 patients.

Analysis will be based on the intention-to-treat principle. Baseline demographic and clinical variables are summarised for each arm of the study. Descriptive summaries of the distributions of continuous baseline variables will be presented in terms of percentiles (e.g. median, 25th and 75th percentile), while discrete variables will be summarised in terms of frequencies and percentages. The statistical comparisons of the treatment arms with respect to the primary endpoint will be performed using the Wilcoxon rank-sum test as the principal analytic tool. All p-values will be 2-sided. Comparisons will be between the sodium nitrite-treated and placebo control-treated group for the primary and secondary outcomes.

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Monitoring

A trial steering committee composed of three independent experts in the fields of: pharmacology, interventional cardiology and clinical trials along with the investigators will monitor the study. This will include a lay member to focus on patient issues. This committee will meet before patient recruitment and then annually to assess safety, feasibility or any other arising problems (e.g. with recruitment) and their recommendations will be followed. In addition an independent Data and Safety Monitoring Committee (DSMC) will monitor patients' safety and treatment efficacy data while the trial is ongoing. This committee is independent of the sponsor and investigators, and has no competing interests. The DSMC will meet prior to initiation of the clinical study, after the recruitment of 10 patients and then at 3 monthly intervals. The DSMC will have access to unblinded patient data. If a serious concern with the safety of the patients in the trial would arise, the DSMC may recommend early termination of the study.

Ethical considerations and dissemination

Ethical considerations

The important ethical considerations concern 1) consent in the acute setting, 2) the risk of the IMP itself and 3) any delay in door to balloon time.

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1) Seeking informed consent for clinical research from patients suffering AMI is an ethical challenge owing to the medical condition of the patients, the emergency situation, and the limited time available. There is no obvious solution to the particular difficulties of informed consent in this situation, and so previously patients have been enrolled in AMI trials on the basis of more or less comprehensive consent procedures. We have addressed this by excluding patients who are unconscious, critically unstable (cardiogenic shock) or deemed unable to consent (pain, distress, language) and by also providing a clear and concise summary sheet shown to the patient during the consent process.

2) The risks from the intervention are low. Sodium and nitrite are endogenously-occurring ions with no immunological potential, therefore there is no risk of an allergic reaction. The small volume of 1.8micromol in 10 ml of saline given over 30 seconds is very unlikely to pose any problems.

3) The delivery of the IMP (sodium nitrite) down the coronary artery will lead to a small delay in balloon inflation, however any possible delay has been minimised by

a) the guidewire may have already restored some epicardial flow before the IMP is delivered

b) the nitrite infusion will be delivered through an over the wire balloon which can be inflated immediately after infusion restoring flow if necessary otherwise an export catheter will be used to aspirate thrombus first

c) the time of the infusion has been kept to 30 seconds meaning at most there should be a delay of 1-2 minutes in the door to balloon time.

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Potential benefits

If the impressive effect of sodium nitrite on reperfusion injury does translate from animal studies to humans, this would result in smaller infarcts and potentially better functional parameters in patients treated with nitrite. If this positive outcome occurs this would pave the way for the future development of this cheap and easily manufactured substance that could be made readily and widely available to the general population in the future.

Dissemination

The study will be performed in agreement with the Declaration of Helsinki and is approved by the local ethics committee (NRES Committee London West London: 11/LO/1500). The study has also been approved by the Medicines and healthcare products Regulatory Agency (MHRA) (EudraCT nr. 2010-022460-12). Data collection will be completed by mid 2013. Primary and secondary analysis will commence immediately after data monitoring is completed, publications will be prepared for submission in late 2013. The results of the trial will be published according to the CONSORT statement. Dissemination of results will focus on publications in peer-reviewed journals, presentations at national/international cardiology meetings and NHS groups. In accordance with recommendations, we have registered NITRITE-AMI with public registries, the United Kingdom Clinical Research Network (Study ID 12117),http://clinicaltrials.gov (NCT01584453) and Current Controlled Trials (International Standard Randomised Controlled Trials No: 38736987).

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Summary

Experimental studies in myocardial I/R injury have demonstrated a protective effect of sodium nitrite but to date no clinical studies have been performed. The NITRITE-AMI study is a 1st in man study assessing the safety and efficacy of intra-coronary nitrite infusion during acute myocardial infarction. The results of the study will set the stage for a larger trial to evaluate the safety and efficacy of sodium nitrite during STEMI.

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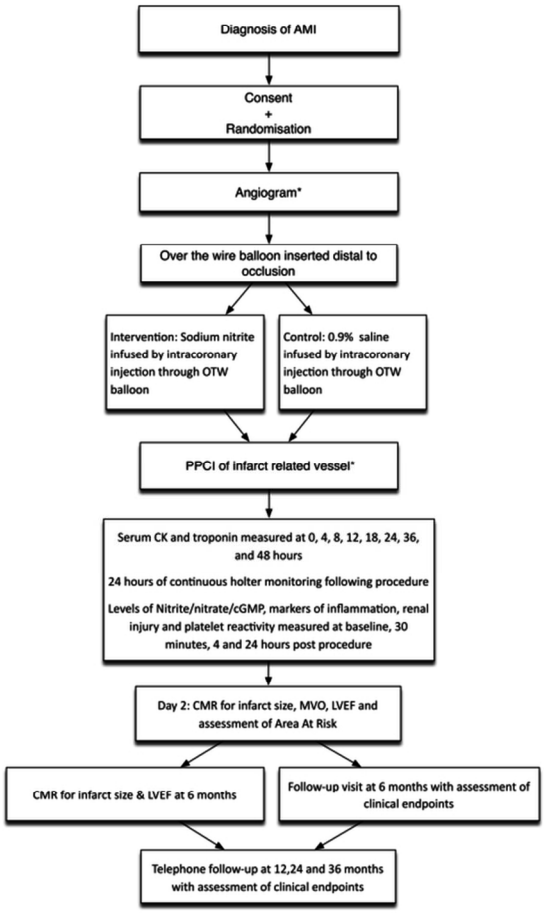
Figure 1. Flow diagram of Study design



AMI: acute myocardial infarction, OTW: Over the wire balloon, PPCI: primary percutaneous coronary intervention, CMR: cardiac magnetic resonance imaging, MVO: microvascular obstruction, LVEF: Left ventricular ejection fraction

90x116mm (300 x 300 DPI)

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