The Effect of Remote Ischemic Conditioning and Glyceryl Trinitrate on Perioperative Myocardial Injury in Cardiac Bypass Surgery Patients: Rationale and Design of the ERIC-GTN Study

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ABSTRACT

Remote ischemic conditioning (RIC) using transient limb ischemia/reperfusion has been reported to reduce perioperative myocardial injury in patients undergoing coronary artery bypass grafting and/or valve surgery. The role of intravenous glyceryl trinitrate (GTN) therapy administered during cardiac surgery as a cardioprotective agent and whether it interferes with RIC cardioprotection is not clear and is investigated in the ERIC-GTN trial (<http://www.clinicaltrials.gov: NCT01864252>). The ERIC-GTN trial is a single-site, double-blind, randomized, placebo-controlled study. Consenting adult patients (age > 18 years) undergoing elective coronary artery bypass grafting $\pm$ valve surgery with blood cardioplegia will be eligible for inclusion. Two hundred sixty patients will be randomized to 1 of 4 treatment groups following anesthetic induction: (1) RIC alone, a RIC protocol comprising three 5-minute cycles of simultaneous upper-arm and thigh cuff inflation/deflation followed by an intravenous (IV) placebo infusion; (2) GTN alone, a simulated sham RIC protocol followed by an IV GTN infusion; (3) RIC + GTN, a RIC protocol followed by an IV GTN infusion; and (4) neither RIC nor GTN, a sham RIC protocol followed by IV placebo infusion. The primary endpoint will be perioperative myocardial injury as quantified by the 72-hour area-under-the-curve serum high-sensitivity troponin T. The ERIC-GTN trial will determine whether intraoperative GTN therapy is cardioprotective during cardiac surgery and whether it affects RIC cardioprotection.

Introduction

Coronary artery disease is one of the leading causes of morbidity and mortality worldwide. For patients with

multivessel coronary artery disease, the treatment of choice is coronary revascularization by coronary artery bypass graft (CABG) surgery. However, due to a number of factors, including the aging population, increased prevalence of comorbidities (such as diabetes mellitus [DM], hypertension [HTN], and obesity), and a growing need for concomitant valve surgery, higher-risk patients are undergoing this surgery, resulting in worse postoperative clinical outcomes.^{1,2} Therefore, new treatment strategies are required to protect the heart and other organs during cardiac surgery so that clinical outcomes can be improved.

In this regard, cardioprotection can be noninvasively elicited by simply inflating a standard blood-pressure (BP)

Ashraf Hamarneh and Vivek Sivaraman contributed equally. This research study was funded by the Rosetrees Trust and the National Institute for Health Research University College London Hospitals Biomedical Research Centre. D.J.H. is supported by a BHF Senior Clinical Research Fellowship (FS/10/039/28270).

The authors have no other funding, financial relationships, or conflicts of interest to disclose.

Figure 1. Patient pathway for the ERIC-GTN trial. Abbreviations: AKI, acute kidney injury; CABG, coronary artery bypass grafting; GTN, glyceryl trinitrate; hs, high sensitivity; ICU, intensive care unit; IV, intravenous; RIC, remote ischemic conditioning.

cuff placed on the upper arm or thigh to induce cycles of brief ischemia/reperfusion, a phenomenon that has been termed remote ischemic conditioning (RIC)^{3,4} and that has been reported to reduce the magnitude of perioperative myocardial injury during cardiac surgery.^{5–7} However, not all studies have reported beneficial effects with RIC on this surrogate endpoint.^{8,9} The potential reasons for this difference in study outcomes are numerous and include factors that are known to have an impact on cardioprotection during cardiac surgery, such as the RIC protocol itself and the presence of comorbidities such as DM and HTN.^{10–12} One other major reason relates to the medications administered during cardiac surgery—these include volatile anesthetics, propofol, and opiates, which have all been reported to influence cardioprotection during cardiac surgery.^{13–15}

In a recent post-hoc analysis, we found that the cardioprotective effect of RIC was abolished in the presence of intraoperatively administered intravenous (IV) glyceryl trinitrate (GTN) therapy.⁷ Furthermore, GTN therapy alone actually reduced the extent of perioperative myocardial injury by 39%, suggesting that in itself intraoperative GTN may be cardioprotective. Intraoperative GTN, a nitric oxide (NO) donor, is administered during cardiac surgery to control systemic BP and vasodilate arterial grafts as and when it is required. There is an extensive preclinical literature supporting the cardioprotective effect of NO,¹⁶ but whether NO is cardioprotective during cardiac surgery has not been tested. As the potential cardioprotective effects

of GTN therapy during cardiac surgery were discovered in a post-hoc analysis,⁷ they need to be confirmed in a prospectively designed, randomized controlled clinical trial. Hence, the Effect of Remote Ischemic Conditioning and Glyceryl Trinitrate on Perioperative Myocardial Injury in Cardiac Bypass Surgery Patients (ERIC-GTN) trial has been designed to determine whether intraoperatively administered IV GTN is cardioprotective during cardiac surgery and to investigate the interaction between RIC and IV GTN in their cardioprotective effects.

Study Design

The ERIC-GTN study will be a single-site, randomized, placebo-controlled clinical trial designed to investigate the cardioprotective effects of RIC and GTN therapy during cardiac surgery (<http://www.clinicaltrials.gov> identifier NCT01864252; Figure 1). The study received ethical approval from the National Health Service Research Ethics Committee, will conform to the spirit and the letter of the Declaration of Helsinki, and will be conducted in accordance with the principles of Good Clinical Practice under the oversight of University College London Hospital. All participants will provide written informed consent.

Study Participants

Patient inclusion criteria will be as follows: adult patients (age ≥18 years) undergoing on-pump cardiac surgery with

Table 1. Study Participants

Inclusion criteria
Age >18 y
Patient undergoing on-pump CABG ± valve surgery
Exclusion criteria
History of cardiogenic shock or cardiac arrest during the current admission
Pregnancy
Significant PAD affecting the upper limbs
Significant hepatic impairment (bilirubin >20 mmol/L, INR >2.0)
Significant pulmonary disease (FEV1 < 40% predicted)
Renal failure with a GFR <30 mL/min/1.73 m ²
Allergy to GTN
Abbreviations: CABG, coronary artery bypass grafting; FEV1, forced expiratory volume; GFR, glomerular filtration rate; GTN, glyceryl trinitrate; INR, international normalized ratio; PAD, peripheral arterial disease.

blood cardioplegia. Patient exclusion criteria are given in full in Table 1. Baseline characteristics for the first 114 patients recruited into the study are shown in Table 2.

Study Outcomes

The primary endpoint of the study will be perioperative myocardial injury as quantified by the 72-hour area-under-the-curve (AUC) serum high-sensitivity troponin T (hs-TnT), measured from serum hs-TnT samples obtained preoperatively, 6, 12, 24, 48, and 72 hours postsurgery. The secondary endpoints will include maximum inotrope score in the 72-hour perioperative period,¹⁷ ventilatory requirements (this will include ventilation duration, fraction of inspired oxygen [FiO₂] requirement and duration of endotracheal intubation, and re-intubation rates), atrial fibrillation (its incidence, the need for treatment, and paroxysmal vs persistent), acute kidney injury grades 1, 2, or 3 within 72 hours of surgery (International Kidney Disease: Improving Global Outcomes¹⁸), and length of intensive care unit and hospital stay.

Randomization

Randomization will be conducted via a secure website (Sealed Envelope) by a named, unblinded research investigator who will also administer the study protocols. The patients, anesthetists, cardiac surgeons, intensive care unit, ward staff, and the research investigators collecting and analyzing the data all will be blinded to the treatment allocation.

Study Interventions

Patients will be assigned randomly to 1 of 4 treatment groups:

1. RIC alone (n = 65): Following anesthetic induction, the RIC protocol will applied, and once the patient

Table 2. Baseline Characteristics for 114 Patients Recruited So Far

Characteristic (N = 114)	Value
Sex	
M	87 (76.3)
F	27 (23.7)
Age, y, mean ± SD	69 ± 11
Comorbidities	
DM	30 (26.3)
Hypercholesterolemia	60 (52.6)
HTN	69 (60.5)
Previous MI	25 (21.9)
Previous PCI	23 (20.2)
Previous CABG	2 (1.8)
Previous TIA/stroke	8 (7.0)
AF	12 (10.5)
PVD	2 (1.8)
Smoking history	
Ever smoked	73 (65.8)
Ex-smoker	56 (51.9)
NYHA class	
I	20 (24.4)
II	34 (41.5)
III	27 (32.9)
IV	1 (1.2)
CCS class	
I	15 (24.6)
II	28 (45.9)
III	17 (27.9)
Medications	
ASA	74 (64.9)
β-Blocker	75 (65.8)
CCB	34 (29.8)
Nitrates	34 (29.8)
ACEI/AT ₂ receptor antagonist	69 (60.5)
Insulin	6 (5.3)
Clopidogrel/prasugrel	34 (29.8)
Abbreviations: ACEI, angiotensin-converting enzyme inhibitor; AF, atrial fibrillation; ASA, aspirin; AT ₂ , angiotensin II; CABG, coronary artery bypass grafting; CCB, calcium channel blocker; CCS, Canadian Cardiovascular Society; DM, diabetes mellitus; F, female; HTN, hypertension; M, male; MI, myocardial infarction; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; PVD, peripheral vascular disease; SD, standard deviation; TIA, transient ischemic attack. Data are presented as n (%) unless otherwise noted.	

has been transferred to the operating theater, an IV placebo infusion will be administered.

2. GTN alone (n = 65): Following anesthetic induction, the sham RIC protocol will applied, and once the patient has been transferred to the operating theater, an IV GTN infusion will be administered.
3. RIC + GTN (n = 65): Following anesthetic induction, the RIC protocol will applied, and once the patient has been transferred to the operating theater, an IV GTN infusion will be administered.
4. Neither RIC or GTN (n = 65): Following anesthetic induction, the sham RIC protocol will applied, and once the patient has been transferred to the operating theater, an IV placebo infusion will be administered.

The treatment protocols will be applied after anesthesia induction and prior to surgical incision, and they will not prolong the anesthetic time or delay the onset of surgery.

Remote Ischemic Conditioning and Sham Remote Ischemic Conditioning Protocols: For patients randomized to receive the RIC protocol, a standard BP cuff will be placed on the upper arm and another standard BP cuff will be placed on the upper thigh. The cuffs will then be simultaneously inflated to 200 mm Hg and left inflated for 5 minutes, then deflated to 0 mm Hg and left uninflated for 5 minutes. This cycle will be repeated 3 times in total, so that the duration of the RIC protocol will be 30 minutes. If the systolic blood pressure (SBP) is >185 mm Hg, the cuffs will be inflated to 15 mm Hg above the SBP. This RIC protocol is a modification of that used in a previous study.⁷ For patients randomized to receive the sham RIC protocol, the cuffs will be left uninflated for 30 minutes.

Glyceryl Trinitrate Therapy and Placebo: The IV GTN or placebo infusions will be commenced on arrival in the operating theater and prior to surgical incision. The placebo infusion will be normal saline administered at 2 mL/h. The IV GTN infusion (1-mg/mL solution) will be started at 2 mL/h and titrated to between 2 and 5 mL/h (25–85 mcg/min), ensuring the mean arterial pressure is kept between 60 and 70 mm Hg. The GTN and placebo infusions will be continued until the patient is taken off cardiopulmonary bypass (CPB). We will use GTN at a licensed dose and for the prescribed duration of cardiac surgery. The licensed dose of GTN is 25 to 200 mcg/min or 1.5 to 12 mL/h of a 1-mg/mL commercially available solution. In cases of HTN resistant to an increase in the anesthetic agents, and in cases of coronary or graft vasospasm, a GTN infusion will be administered at the discretion of the anesthetist—this will count as a crossover in the placebo group. In patients randomized to the GTN arm, hypotension will be treated using peripheral vasoconstrictors such as phenylephrine or ephedrine. The maximum dose of GTN used in each patient will be recorded.

Surgical Procedure and Anesthetic Techniques

Patients will receive premedication with oral temazepam 10 to 20 mg 1 hour prior to surgery. Anesthesia induction will be achieved with different combinations of midazolam, etomidate, propofol, fentanyl, remifentanyl, and muscle relaxants (rocuronium, vecuronium, pancuronium, or

atracurium). The trachea will be intubated and mechanical ventilation commenced with oxygen with or without air. Anesthesia maintenance will be achieved with propofol alone, volatile agents (isoflurane or sevoflurane) alone, or a combination (volatile agents prior to and after bypass with propofol at bypass). Arterial BP, central venous pressure, leads I and III of the electrocardiogram, and nasopharyngeal temperature will be recorded continuously. Standard nonpulsatile CPB will be employed using a membrane oxygenator and cardiomy suction; following this, all coronary grafts will be constructed during CPB, using blood cardioplegia. Following anastomosis of the grafts and/or valve replacement/repair, CPB will be discontinued and protamine will be used to achieve heparin reversal.

Statistical Analysis

The study was designed following post-hoc analysis of data from a previous clinical study (now published),⁷ in which we found that in patients administered IV GTN at time of CABG surgery, the cardioprotective effect of RIC was abrogated. To ascertain the interaction between GTN and RIC in the ERIC-GTN study, we have designed a study with 4 treatment groups and based our sample size on detecting a difference between any pairs of the treatment groups. If we assume that the AUC is approximately normally distributed, with 80% power, a 5% 2-sided significance level, and an AUC SD of 21.4 µg/L, then with 50 patients in each group we can observe a difference of ≥12 µg/L in the AUC. Taking into consideration a 5% dropout rate and a 10% crossover rate from the placebo arm to the GTN arm for safety reasons, we anticipate recruiting 260 patients in total, with 65 in each group of the GTN arm and 65 in each group of the placebo arm.

The primary outcome will be the AUC of hs-TnT. We will calculate this by measuring the hs-TnT at 6 time points (0, 6, 12, 24, 48, and 72 hours) and plotting the mean values of all patients in a group on a graph against time. The AUC will then be determined. If the data are approximated by a normal distribution, then a regression model will be used for the analysis. A binary term for each treatment group will be included in the model, and an interaction term (the multiplication of the binary group variables) will also be included. An *F* test will be performed to assess the statistical significance of the interaction term as the primary outcome analysis. If the assumptions of the linear regression model are not met by the data, then the rank-based method of Hettmansperger and Elmore will be used.¹⁹ In this case, a linear regression where only the indicators for each treatment (not the interaction) will be used and an analysis of variance will be performed on the ranks of the resulting residuals. The *F* test on the interaction term in this analysis of variance will be used to assess statistical significance. The level of significance for all analyses is *P* < 0.05.

Any patients in the placebo arm who receive GTN due to safety concerns will be included in the analysis as crossovers in a per-protocol analysis. The primary analysis will be by intention to treat.

For binary outcome variables, a logistic regression model will be used that includes a binary term for each treatment

group and an interaction term. For variables with multiple ordered categories, an ordered logistic model will be used. Time-to-event analyses (secondary clinical endpoints), based on all available follow-up data, will be performed with the use of a Cox regression model including a binary term for each treatment group and an interaction term. A sensitivity analysis will be performed for sensitivity to missing values; for example, using multiple imputation under the missing at random assumption (MAR) and investigating the clinically plausible missing values for patients lost to follow-up. We will follow the recent guidelines suggested by White et al.²⁰ All statistical analyses will be performed using Stata version 12.1 statistical analysis software (StataCorp LP, College Station, TX).

Data Management and Funding

University College London is the sponsor of this trial. Data will be collected using a paper case report form and entered onto the Web-based electronic RedCap database.²¹ An independent data monitoring committee will be installed to monitor the progress of the study as well as any safety concerns. All expected or unexpected adverse events will be reviewed continuously and be reviewed by the investigators, the sponsor, and the independent data monitoring committee according to the sponsor's regulations. This work is supported by the RoseTrees Trust and the National Institute for Health Research University College London Hospitals Biomedical Research Centre.

Discussion

In the ERIC-GTN study, we intend to investigate the roles of RIC and GTN as cardioprotective strategies during cardiac surgery. A number of small clinical studies have reported that RIC can reduce the extent of perioperative myocardial injury in patients undergoing CABG surgery,^{5–7} but not all studies have reported this beneficial effect with RIC on this biochemical marker.^{8,9} The reasons for this are many and relate to a number of factors, including the RIC protocol, the setting of CABG surgery itself, comorbidities, and concomitant medications that are known to interfere with endogenous cardioprotective strategies.¹⁰ For example, propofol has been shown to abrogate the cardioprotective effect of RIC in the setting of CABG surgery,^{13,14} although this was a small study comprising 72 patients in total with 4 study groups.

Another medication administered during cardiac surgery that has the potential to interfere with RIC cardioprotection may be GTN. In a recent study investigating the effect of RIC on short-term clinical outcomes following cardiac surgery, we found from a post-hoc analysis of those patients who did not receive RIC that those patients administered intraoperative IV GTN during cardiac surgery sustained a 39% reduction in perioperative myocardial injury as measured by the 72-hour AUC of hs-TNT.⁷ These data suggested that intraoperative GTN in itself may be cardioprotective in patients undergoing cardiac surgery. However, a similar post-hoc analysis by Kleinbongard et al²² failed to find this beneficial effect with GTN in patients undergoing cardiac surgery with crystalloid cardioplegia.

Therefore, the role of intraoperative GTN as a potential cardioprotective therapy during cardiac surgery needs to be investigated in a prospective randomized controlled clinical trial. This is the first objective of the ERIC-GTN trial, which will investigate whether intraoperative GTN can reduce the magnitude of perioperative myocardial injury during cardiac surgery.

The second objective of the ERIC-GTN study will be to investigate whether administering an IV GTN infusion during surgery affects the cardioprotective efficacy of RIC. In our prior study, we found that the cardioprotective effect of RIC was abolished in those patients given IV GTN during cardiac surgery.⁷ Whether this was because RIC was unable to confer additional cardioprotection over that provided by the GTN itself or whether it was due to GTN diminishing RIC cardioprotection will also be investigated in the ERIC-GTN study.

Glyceryl trinitrate is a nitrate that has been in use for more than 100 years for the treatment of angina. It is also licensed for use in cardiac surgery at infusion rates of 25 to 200 mcg/min to lower systemic BP, and its vasodilatory actions are used to maintain graft patency. Nitric oxide released by GTN is known to have several beneficial effects on the cardiovascular system, including protecting the heart against acute ischemia-reperfusion injury when given exogenously or as a mediator of endogenous cardioprotective strategies such as ischemic conditioning.^{16,23} However, the results concerning the involvement of NO in RIC have been investigated using pharmacological inhibitors of NO synthase (NOS) and have produced conflicting results.^{24,25} The contrasting findings may be because NO is not only synthesized from NOS, but it is also formed by nonenzymatic processes, especially during ischemia, and so the failure of NOS antagonists to block cardioprotection does not necessarily rule out its role.²⁶ Nitric oxide has also been demonstrated to be a potential blood-borne cardioprotective factor in form of circulating nitrite.²⁷ In this study, Rassaf et al provided evidence from both murine and human models of RIC, that circulating nitrite derived from shear stress-dependent stimulation of endothelial NOS in the RIC-treated limb may contribute to cardioprotection. Intriguing experimental data have shown that exogenous NO administered during RIC abrogated the latter's infarct-limiting effect, and this was attributed to inhibition of the neural pathway required for RIC cardioprotection.²⁸ However, in our prior clinical study, as in the proposed ERIC-GTN study, as GTN was given on arrival in the operating theater after the RIC protocol had been completed, it probably did not have an effect on the neural pathway required for RIC cardioprotection.

A potential limitation of our study is that we will not be monitoring either the depth of anesthesia or cerebral oxygenation intraoperatively, and as such we will not be able to adequately control the depth of anesthesia or detect episodes of cerebral hypoxia during the operation.

In summary, the ERIC-GTN study will determine whether GTN administered during cardiac surgery can reduce the magnitude of perioperative myocardial injury and whether its presence diminishes the cardioprotective effect of RIC.

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