Daytime variation of perioperative myocardial injury in cardiac surgery and its prevention by Rev-Erbα antagonism: a single-centre propensity-matched cohort study and a randomised study

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Summary

Background On-pump cardiac surgery provokes a predictable perioperative myocardial ischaemia–reperfusion injury which is associated with poor clinical outcomes. We determined the occurrence of time-of-the-day variation in perioperative myocardial injury in patients undergoing aortic valve replacement and its molecular mechanisms.

Methods We studied the incidence of major adverse cardiac events in a prospective observational single-centre cohort study of patients with severe aortic stenosis and preserved left ventricular ejection fraction (>50%) who were referred to our cardiovascular surgery department at Lille University Hospital (Lille, France) for aortic valve replacement and underwent surgery in the morning or afternoon. Patients were matched into pairs by propensity score. We also did a randomised study, in which we evaluated perioperative myocardial injury and myocardial samples of patients randomly assigned (1:1) via permuted block randomisation (block size of eight) to undergo isolated aortic valve replacement surgery either in the morning or afternoon. We also evaluated human and rodent myocardium in ex-vivo hypoxia–reoxygenation models and did a transcriptomic analysis in myocardial samples from the randomised patients to identify the signalling pathway(s) involved. The primary objective of the study was to assess whether myocardial tolerance of ischaemia–reperfusion differed depending on the timing of aortic valve replacement surgery (morning *vs* afternoon), as measured by the occurrence of major adverse cardiovascular events (cardiovascular death, myocardial infarction, and admission to hospital for acute heart failure). The randomised study is registered with ClinicalTrials.gov, number NCT02812901.

Findings In the cohort study (n=596 patients in matched pairs who underwent either morning surgery [n=298] or afternoon surgery [n=298]), during the 500 days following aortic valve replacement, the incidence of major adverse cardiac events was lower in the afternoon surgery group than in the morning group: hazard ratio 0·50 (95% CI 0·32–0·77; p=0·0021). In the randomised study, 88 patients were randomly assigned to undergo surgery in the morning (n=44) or afternoon (n=44); perioperative myocardial injury assessed with the geometric mean of perioperative cardiac troponin T release was significantly lower in the afternoon group than in the morning group (estimated ratio of geometric means for afternoon to morning of 0·79 [95% CI 0·68–0·93; p=0·0045]). Ex-vivo analysis of human myocardium revealed an intrinsic morning–afternoon variation in hypoxia–reoxygenation tolerance, concomitant with transcriptional alterations in circadian gene expression with the nuclear receptor Rev-Erbα being highest in the morning. In a mouse Langendorff model of hypoxia–reoxygenation myocardial injury, *Rev-Erbα* gene deletion or antagonist treatment reduced injury at the time of sleep-to-wake transition, through an increase in the expression of the ischaemia–reperfusion injury modulator CDKN1a/p21.

Interpretation Perioperative myocardial injury is transcriptionally orchestrated by the circadian clock in patients undergoing aortic valve replacement, and Rev-Erbα antagonism seems to be a pharmacological strategy for cardioprotection. Afternoon surgery might provide perioperative myocardial protection and lead to improved patient outcomes compared with morning surgery.

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Introduction

On-pump cardiac surgery is associated with predictable myocardial ischaemia–reperfusion.[1–3] The consequent perioperative myocardial injury is associated with poor clinical outcomes such as left ventricular systolic impairment, the onset of heart failure, and short-term, medium-term, and long-term mortality.[2,4] Despite the emergence of trans-catheter interventions, such as trans-aortic valve implantation, the number of high-risk patients undergoing cardiac surgery is increasing

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Research in context

Evidence before this study

We searched PubMed and MEDLINE from inception until June 30, 2015, using the search terms "circadian", "variation OR dependence", "myocardial infarction size", and "patients". We found nine original articles on this topic. Although studies on circadian gene knockout and mutant mice revealed a biorhythm in myocardial ischaemia–reperfusion tolerance, these findings did not translate into a feasible strategy to confer cardioprotection in patients. Moreover, mixed results have been reported regarding the clinical consequences of such a biorhythm in patients undergoing acute myocardial infarction.

Added value of this study

The results of this study show that patients undergoing aortic valve replacement surgery in the afternoon display lower perioperative myocardial injury and postoperative morbidity than those operated on in the morning. Perioperative myocardial injury is transcriptionally regulated by the circadian clock, with Rev-Erbα antagonism emerging as a pharmacological strategy for cardioprotection.

Implications of all the available evidence

A clinically relevant biorhythm exists in myocardial ischaemia–reperfusion tolerance. Through consideration of the timing of surgery, it could be possible to improve outcomes in these patients, with afternoon surgery providing perioperative myocardial protection and better outcomes.

substantially. This increase is due to the ageing population, the rising prevalence of associated comorbidities (eg, diabetes mellitus and renal failure), and an increase in the number of patients with the usual indication for combined surgery (ie, coronary artery bypass graft [CABG] with concomitant valve and/or aortic surgery).[5,6] These high-risk patients are especially susceptible to perioperative myocardial injury,[7] resulting in worsened clinical outcomes following surgery; therefore, novel cardioprotective strategies must be explored, since the most recent approaches such as remote ischaemic preconditioning have failed to demonstrate success in the clinic.[1,5,6]

Cardiovascular diseases show diurnal variation, with a higher incidence of ST-segment elevation myocardial infarction (STEMI) in the early morning than in the evening.[8] Although studies of circadian gene-knockout and mutant mice argue for a biorhythm in myocardial ischaemia–reperfusion tolerance,[8–10] whether or not such a biorhythm, leading to meaningful differences in outcomes, exists in human beings remains unclear because of conflicting reports in the context of STEMI.[11–15] Larger infarct sizes or a higher incidence of heart failure secondary to STEMI occurring in the early morning than later in the day have been reported in several studies.[11–14] However, in the largest (n=1099) multicentre study,[15] Ammirati and colleagues were unable to show an effect of the time of the day on STEMI burden. In view of the interplay between ageing and the circadian clock,[16] whether or not a time-of-the-day variation in perioperative myocardial injury exists in the ageing population undergoing cardiac surgery is unknown.

To assess if cardiac surgery done in the morning or afternoon has different clinical consequences, we studied the incidence of major cardiac outcomes in a large prospective cohort of patients who underwent scheduled aortic valve replacement. We also tested the effect of time of the day on perioperative myocardial injury in patients scheduled for aortic valve replacement and randomly assigned to undergo surgery either in the morning or the afternoon. We also did a transcriptomic analysis in myocardial samples from these randomised patients to identify the signalling pathway(s) involved. Among the most regulated genes, the Rev-Erbα nuclear receptor was tested as a potential pharmacological target for cardioprotection.

Methods

Study design and participants

The cohort study population consisted of all consecutive patients (aged ≥18 years) with severe aortic stenosis and preserved left ventricular ejection fraction (>50%) referred to our cardiovascular surgery department at Lille University Hospital (Lille, France) for aortic valve replacement (with or without coronary artery bypass graft) between Jan 1, 2009, and Dec 31, 2015. Patients with another notable valvular disease, a medical history of previous cardiac surgery, or congenital heart diseases were excluded. The ethics committee of our institution approved the cohort protocol. Written informed consent was obtained from all patients before inclusion in this cohort.

For the randomised study, patients (aged ≥18 years) undergoing isolated aortic valve replacement surgery for aortic valve stenosis with preserved left ventricular ejection fraction in our hospital (Lille University Hospital Hospital Lille, Lille, France) were enrolled between Jan 1, 2016, and Feb 28, 2017. Enrolment and randomisation were done the day before the patients' scheduled surgery. Patients with diabetes mellitus or renal insufficiency (serum creatinine level >150 μM) were excluded to maximise perioperative risk homogeneity. Patients with a history of atrial fibrillation or atrial flutter were also excluded, to allow postoperative atrial fibrillation to be assessed as an endpoint in

all patients. Patients with substantial coronary stenosis (>50%) on the preoperative angiogram were excluded. All exclusions were done before randomisation. The randomised study was done in accordance with the Declaration of Helsinki (revised version, 1996), the European Guidelines for Good Clinical Practice (version 11, July, 1990), and French laws. The ethics committee of our institution approved the study protocol. Written informed consent was obtained from all patients before inclusion.

Randomisation and masking

For the randomised study, patients were randomly assigned (1:1) to morning or afternoon surgery by restricted permuted block randomisation, with a block size of eight. Randomisation was done on the day before the patients' scheduled surgery. The code sequence was computer generated and kept in sealed envelopes at a central location (Direction de la Recherche Clinique, Lille University Hospital, Lille, France) by non-medical staff not involved in the study. For each patient randomised, the next available code was used. Senior and junior staff cardiologists not involved in the perioperative treatment or analysis enrolled participants. After patient consent was obtained, these staff cardiologists opened the envelope to assign the patients to the morning or afternoon schedule of the surgery theatre. Patient inclusion did not modify the type of intervention, which was chosen before enrolment. Patient inclusion did not change the daily practices since the two senior surgeons involved in this randomised study operate twice in every working day allocated to scheduled surgery, with one patient being operated on in the morning and one in the afternoon by the same medical team. Therefore, cardiac surgeons and intensive-care physicians were kept unaware of the inclusion of their patients in the study until hospital discharge. Anaesthesia, cardiopulmonary bypass, cardioplegia, and surgical procedures were done without any modification of the customary routine in our hospital.

Procedures

Patients in both the cohort study and randomised study underwent aortic valve replacement either in the morning or in the afternoon by one of the four senior surgeons who operated twice every working day: the same surgical team did both morning and afternoon surgery on the same day. Anaesthaesia, cardiopulmonary bypass, cardioplegia, and surgical procedures were done according to standard guidelines. Anaesthesia was induced with intravenous sufentanil (1·0–1·5 µg/kg) and propofol (0·5–1·5 mg/kg), and maintained with end-tidal sevoflurane (1·7% of the expired fraction). Surgery was done using normothermic cardiopulmonary bypass and repeated antegrade cold crystalloid-blood cardioplegia. Right atrial biopsy was obtained during pulmonary bypass preparation as previously described.[17]

In the cohort study, propensity score matching was used to select comparable morning and afternoon groups. Postoperative clinical outcome was assessed in these 1:1 matched populations.

In the randomised study, which was done to minimise confounding, eligible patients were randomly assigned (1:1) to undergo surgery either in the morning or the afternoon. Right atrial biopsies were obtained from the first 22 patients randomly assigned in the two morning and afternoon groups. Collection of such biopsies is routine practice in our hospital and therefore did not affect the randomisation concealment of the surgical team.

For the cohort study, all patients underwent clinical follow-up during the 500 days after surgery. Data were obtained from medical records and interviews with the general practitioners of the patients. Recorded clinical events were: major adverse cardiac events, including cardiovascular death, myocardial infarction, and admission to hospital for acute heart failure. Each endpoint was centrally reviewed by two independent cardiologists who were masked to the time of the day that the patient had surgery. In case of disagreement, the endpoint was discussed with a third cardiologist.

In the randomised study, perioperative myocardial injury and postoperative complications until hospital discharge were recorded.[18] Recorded clinical events were obtained from medical records and were: postoperative atrial fibrillation, requirement for inotropic support, and major adverse cardiac events including cardiovascular death and perioperative myocardial infarction.[18] These events were recorded by senior and junior staff cardiologists not involved in the perioperative treatment or analysis. Perioperative myocardial injury was estimated by the area under the curve (AUC) for cardiac troponin T concentrations, calculated according to the trapezoidal rule.[18] Venous blood samples were drawn from each patient preoperatively on the day before surgery and postoperatively at 6, 9, 12, 24, 48, and 72 h and analysed for serum cardiac troponin T concentration. No patients were excluded for missing data.

The experiments on atrial samples from human tissue were done as previously described[19] and detailed in the appendix (p 2). Atrial myocardial samples were obtained via biopsy from patients in the morning and the afternoon. Atrial trabeculae were sequentially exposed to hypoxic and reoxygenation conditions. Contraction recovery from hypoxia–reoxygenation challenge was compared between the morning and afternoon group samples.

See Online for appendix

For the transcriptomic study, total RNA was extracted from human and mouse tissues and analysed on Agilent SurePrintG3 HumanGeneExpression 8x60Kv2 (Agilent, Santa Clara, CA, USA) or Affymetrix MoGeneST2 assays (Affymetrix-ThermoFisher, Santa Clara, CA, USA), respectively. High-density oligonucleotide arrays were used to identify differentially expressed genes in atrial samples from patients operated on in the morning matched with patients operated on in the afternoon. Data

	Morning surgery (n=298)	Afternoon surgery (n=298)	p value
Demographics			
Age, years	71 (10)	71 (10)	0·88
Male sex	162 (54%)	160 (54%)	0·93
Body-mass index, kg/m²	28 (6)	29 (6)	0·38
Risk factors and comorbidities			
Diabetes mellitus	85 (29%)	84 (28%)	0·92
Hypertension	187 (64%)	193 (65%)	0·83
Cardiac status			
Serum creatinine concentration, μM	80 (71–96)	80 (71–99)	0·78
New York Heart Association class	2·2 (0·6)	2·2 (0·6)	0·96
Left ventricular mass index, g/m$^{2·7}$	49 (15)	50 (16)	0·36
Left ventricular ejection fraction, %	62% (8)	62% (8)	0·67
Left main artery ≥50% stenosis	48 (16%)	51 (17%)	0·83
Three-vessel coronary artery disease	46 (15%)	42 (14%)	0·73
Euroscore II, %	1·78% (1·32)	1·76% (1·32)	0·87
STS morbidity risk, %	12·1% (6·5)	12·1% (6·6)	0·99
Preoperative medication			
Aspirin	77 (26%)	72 (24%)	0·50
Beta blockers	74 (25%)	68 (23%)	0·42
Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers	164 (55%)	160 (54%)	0·74
Statins	125 (42%)	131 (44%)	0·62
Diuretics	134 (45%)	133 (44%)	0·93
Surgery characteristics			
Cardiopulmonary bypass duration, min	93 (32)	92 (31)	0·83
Aortic cross-clamp duration, min	74 (30)	74 (27)	0·90
Concomitant coronary artery bypass graft	81 (27%)	70 (24%)	0·40
Number of coronary artery bypass graft anastomoses	1·37 (0·60)	1·36 (0·61)	0·84
Biological prosthesis	245 (82%)	244 (82%)	0·99
Prosthesis diameter, mm	22 (2)	22 (2)	0·32
Cardiac defibrillation	15 (5%)	20 (7%)	0·49
Surgeon A/B/C/D	75/94/63/69	71/101/57/66	0·88

Data are mean (SD) or n (%). p values calculated by log-rank test. STS=Society of Thoracic Surgeons.

Table 1: **Baseline characteristics and intraoperative events in the 596 patients in the cohort study**

For the **ICD-10 codes** see http://apps.who.int/classifications/apps/icd/icd10online/

were analysed using the Genespring software suite, version 12.0.

Experiments on ex-vivo mouse heart models were approved by the local committee Direction Départementale des Services Vétérinaires-Nord-Pas-de-Calais-Lille (Lille, France). Mouse hearts were explored in the isolated Langendorff model at the sleep-to-wake transition (Zeitgeber time ZT12) and the wake-to-sleep transition (ZT0), as described.[20] Hearts were subjected to global ischaemia for 35 min, reperfused for 45 min, and infarct sizes measured.[21] Modulation of ischaemia–reperfusion tolerance with the Rev-Erbα antagonist SR8278 (Sigma, St Louis, MO, USA)[22] was tested in isolated perfused hearts after in-vivo administration (25 mg/kg [or dimethyl sulfoxide vehicle] given intraperitoneally at ZT8 and ZT11). An extended methods section is available in the appendix (pp 2–4).

Outcomes

The primary objective of the cohort study was to assess whether myocardial tolerance of ischaemia–reperfusion differed depending on the timing of aortic valve replacement surgery (morning *vs* afternoon), as measured by the occurrence of major adverse cardiovascular events (cardiovascular death, myocardial infarction, and admission to hospital for acute heart failure). The primary objective of the randomised study was to assess whether myocardial tolerance of ischaemia–reperfusion differed depending on the timing of aortic valve replacement surgery (morning *vs* afternoon), as measured by the severity of perioperative myocardial injury (defined in the procedures section). Cardiovascular death was defined according to the International Classification of Diseases, tenth revision (ICD-10 codes I00–I78). Myocardial infarction included both perioperative myocardial infarction (type 5) and myocardial infarction after cardiac surgery.[5] Perioperative myocardial infarction (type 5) was defined by the association on the days following surgery of high concentrations of high-sensitivity troponin T in serum (>10× coefficient of variation of 10% for fourth-generation troponin T) with new pathological Q-waves, left bundle-branch block, or abnormal left ventricular wall motion on trans-thoracic echocardiogram at discharge. Post-surgical myocardial infarction was defined as an increase in serum high-sensitivity cardiac troponin T concentration (measured using Elecsys Troponine T-hs, Roche Diagnostics, Meylan, France) from baseline to at least twice the upper limit of normal, together with evidence of myocardial ischaemia, such as angina symptoms or ECG changes, including persistent ST-segment or T-wave changes or new Q-waves. Admission to hospital for acute heart failure was defined as hospital admission for dyspnoea, peripheral oedema, or both, with elevated blood natriuretic peptide adjusted for age and renal function.

Statistical analysis

For the cohort study, propensity score matching was used to select comparable groups of patients. The propensity matching score was estimated by multivariable logistic regression. In the regression model, time of day of surgery was the dependent variable. The independent variables were chosen because of their prognostic significance in the previously published literature[4–6,18] or our cohort (appendix p 10) and were: age, sex, serum creatinine concentration, medical history of diabetes mellitus, indexed left ventricle mass, Euroscore II, concomitant coronary bypass graft, and aortic cross-clamping duration. After estimation of the propensity score,

patients in the morning group were matched in a 1:1 ratio to those in the afternoon group. The optimal matching algorithm with a caliper size of within 1% of the estimated propensity score was used to construct a matched-paired sample.

For the randomised study, the sample size calculation for patients was done according to the previous study by Chiari and colleagues.[3] Our hypothesis was that there would be a 35% (SD 58%) relative difference in troponin release (troponin T AUC) between the afternoon and morning group, which meant that a sample size of 44 patients in each group was needed to get a power of 80% for a significance level of 5% with a two-tailed test.

The number of ex-vivo experiments on human atrial tissue (n=22 per group) was calculated a priori based on previous data.[19] We postulated that there would be a 20% (SD 20%) relative difference in contraction recovery between the afternoon and morning groups, hence requiring a sample size of 22 experiments in each group to obtain 90% power for a significance level of 5% with a two-tailed test.

Continuous variables with a Gaussian distribution are provided as mean (SD) or mean (standard error of the mean [SEM]) as specified. Continuous variables with no Gaussian distribution are given as median (IQR). Categorical variables are given as the number (percentage) of patients with the respective attribute. Bivariate comparisons were performed using the *t* test for normally distributed continuous variables or the Mann-Whitney *U* test for variables not normally distributed. Bivariate comparisons of categorical variables were done with the χ^2 test.

For multiple comparisons of normally distributed variables between more than two groups, one-way analysis of variance (ANOVA) was used with post-hoc *t* tests and Bonferroni corrections.

For time-to-event variables, the survival functions were estimated with the Kaplan-Meier method and compared by log-rank. Cox's proportional hazard regression was used to obtain hazard ratios (HRs).

AUC values for serum cardiac troponin T concentrations were log-transformed before comparison, and then back-transformed for presentation as the geometric mean (95% CI).

The effect of the time of the day on perioperative myocardial injury—ie, log (troponin AUC)—was adjusted to aortic cross-clamping duration and serum creatinine level by a multivariate linear regression model using no variable selection.

Two-way ANOVA for repeated measure (circadian effect×reoxygenation time) was used to assess the respective effects of time-of-the-day, reoxygenation duration, and their interaction on atrial trabeculae contractile recovery after ischaemia. This ANOVA test was done after checking that data were normally distributed with constant variance. Post-hoc *t* tests were used with Bonferroni corrections.

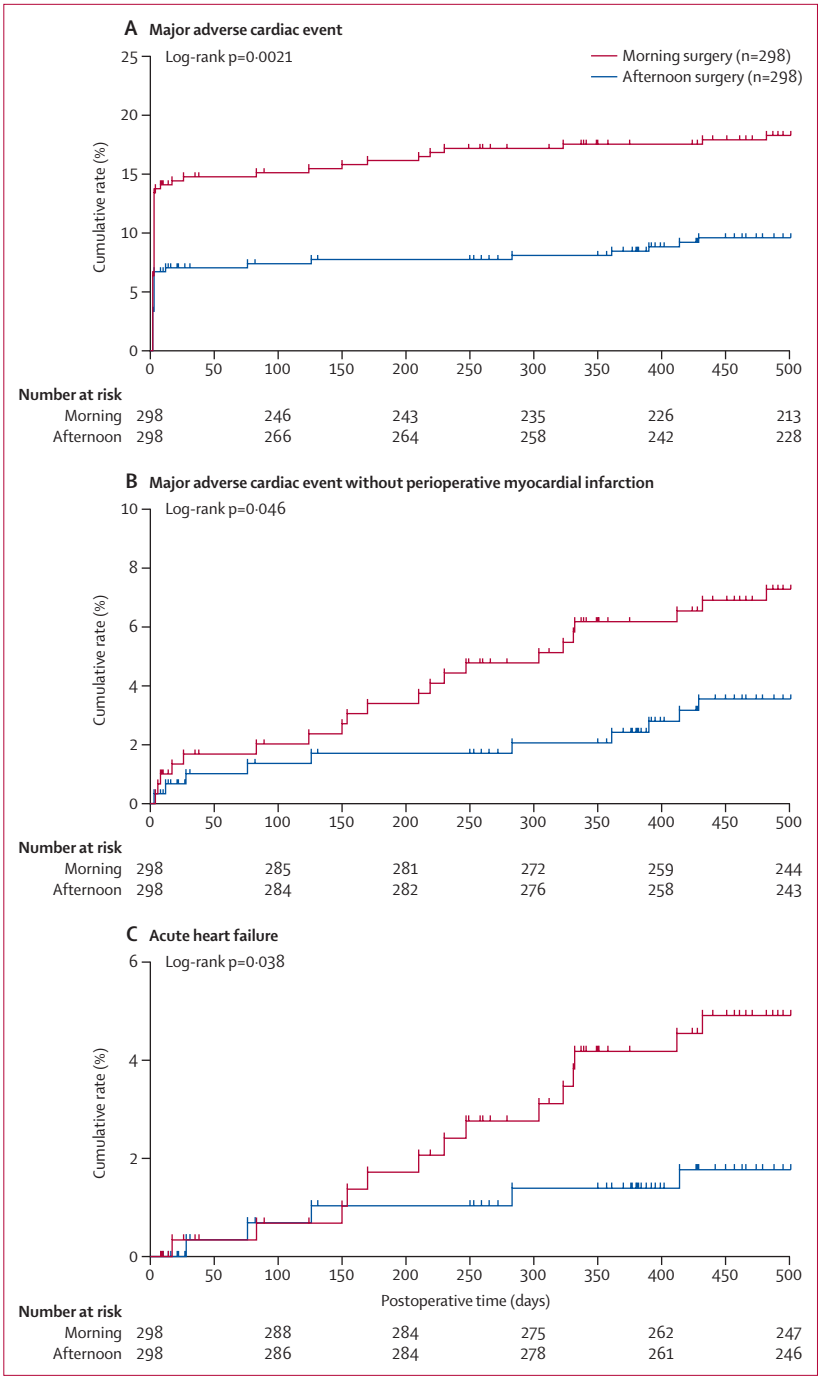


Figure 1: **Cardiovascular events after aortic valve replacement surgery according to time of the day of surgery in the matched cohort population**

(A) Major adverse cardiac events (ie, cardiovascular death, myocardial infarction, and acute heart failure). (B) Major adverse cardiac events without perioperative myocardial infarction. (C) Acute heart failure. p values were calculated by log-rank test.

A value of $p<0·05$ was judged to be statistically significant. All analyses were done using SAS version 9.3.

The randomised study is registered with ClinicalTrials.gov, number NCT02812901.

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. DM and BS had full access to all the data in the study and had final responsibility for the decision to submit for publication.

	Morning surgery (n=298)	Afternoon surgery (n=298)	HR (95% CI) for time-of-day effect pm *vs* am	p value
Death during hospital stay	4 (1%)	2 (0·5%)	NA	0·42
Perioperative myocardial infarction	40 (13%)	19 (6%)	NA	0·0048
Left ventricular ejection fraction at discharge, %	56% (9)	58% (9)	NA	0·0109
Duration of hospital stay, days	11 (10–13)	12 (10–14)	NA	0·53
Major adverse cardiac event	54 (18%)	28 (9%)	0·50 (0·32–0·77)	0·0021
Major adverse cardiac event without perioperative myocardial infarction	21 (7%)	10 (3%)	0·47 (0·23–0·95)	0·0468
Cardiovascular death	11 (4%)	4 (1%)	0·36 (0·13–1·01)	0·0706
Acute heart failure	14 (5%)	5 (2%)	0·36 (0·15–0·88)	0·0386

Data are n (%), mean (SD), or median (IQR). Major adverse cardiac events included cardiovascular death, myocardial infarction, and admission to hospital for acute heart failure. p values calculated by log-rank test. HR (95% CI) calculated by Cox's proportional hazard regression. HR=hazard ratio. NA=not applicable.

Table 2: **Postoperative events in the 596 patients in the cohort study after 500 days of follow-up**

	Morning surgery (n=44)	Afternoon surgery (n=44)	p value
Baseline demographics			
Age, years	69 (8)	69 (9)	0·87
Male sex	28 (64%)	23 (52%)	0·39
Body-mass index, kg/m²	29 (6)	29 (5)	0·76
Risk factors and comorbidities			
Hypertension	27 (61%)	32 (73%)	0·36
Serum creatinine concentration, μM	79 (16)	83 (18)	0·28
Cardiac status			
New York Heart Association class	2·2 (0·8)	2·1 (0·7)	0·71
Left ventricular mass index, g/m²	116 (20)	115 (17)	0·78
Left ventricular ejection fraction, %	62% (9)	61% (7)	0·66
Preoperative medication			
Aspirin	10 (23%)	13 (29%)	0·63
Beta blockers	11 (25%)	10 (23%)	0·95
Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers	23 (52%)	23 (52%)	1·00
Statins	19 (43%)	21 (47%)	0·85
Diuretics	19 (43%)	20 (45%)	0·90
Preoperative risk scores			
Euroscore II, %	1·1 (0·9–1·5)	1·1 (0·9–2·2)	0·29
Surgery characteristics			
Surgery start time	9:15 (9:05–9:35)	15:10 (14:30–15:30)	..
Cardiopulmonary bypass duration, min	83 (21)	85 (22)	0·70
Aortic cross-clamp duration, min	65 (17)	69 (19)	0·31
Cardiac defibrillation	0	0	..
Surgeon A/surgeon B	22/22	20/24	0·83

Data are mean (SD), n (%), or median (IQR).

Table 3: **Baseline characteristics and intraoperative data of the 88 randomised patients**

Results

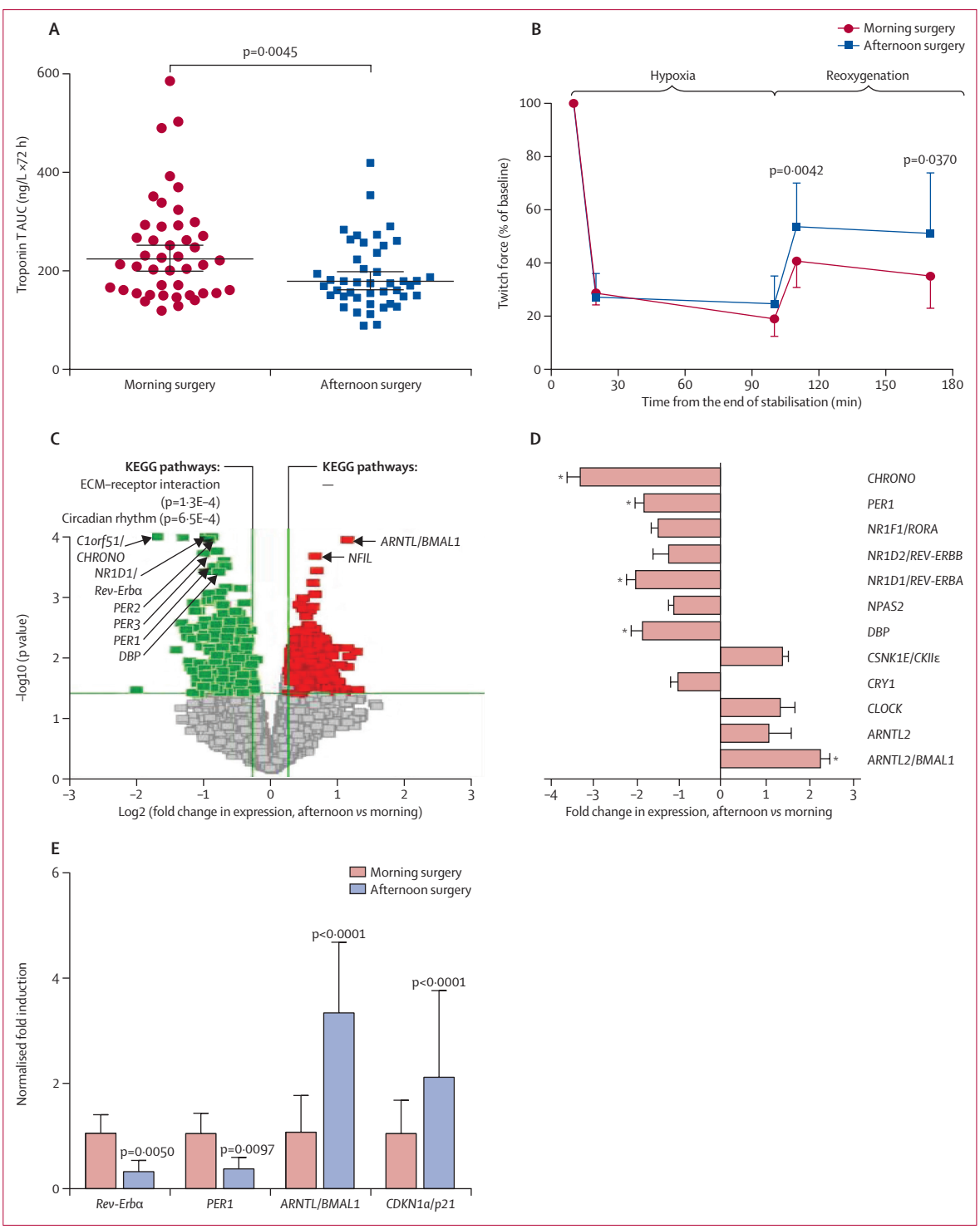
Of the 720 consecutive patients who underwent scheduled aortic valve replacement in our hospital between Jan 1, 2009, and Dec 31, 2015, 350 were operated on in the morning and 298 in the afternoon (appendix p 11). Propensity score matching was used to mitigate the effect of a potential selection bias. In the matched-pair samples, the mean distance in the estimated propensity score was 0·07 (SD 0·15%) and resulted in two well-matched populations of 298 patients with similar preoperative and intraoperative characteristics (table 1). At completion of follow-up (median follow-up 500 days [IQR 480–500]), the frequency of major adverse cardiac events was lower in the afternoon group than in the morning group (HR 0·50 [95% CI 0·32–0·77], p=0·0021). This finding meant that one major adverse cardiac event was prevented for every 11 patients (95% CI 7–30) operated on in the afternoon (*vs* the morning; figure 1A, table 2). Notably, this decreased relative risk for afternoon patients was the result of decreased incidence of both immediate perioperative myocardial infarction and acute heart failure (figure 1B, 1C, table 2). Medium-term postoperative cardiovascular morbidity remained lower in the afternoon than in the morning patients even after exclusion of perioperative myocardial infarction events (figure 1B) and in the subgroup of patients who underwent isolated aortic valve replacement (n=445; appendix p 12).

In the randomised study, 88 patients scheduled for isolated aortic valve replacement surgery were randomly

Figure 2: **Morning-to-afternoon variation in perioperative myocardial injury, hypoxia–reoxgenation tolerance, and gene-expression profiles in human myocardium**

(A) Perioperative myocardial injury in patients randomly assigned to morning (n=44) and afternoon (n=44) aortic valve replacement. The horizontal line and error bars for each group show the geometric mean (95% CI) of the area under the curve (AUC) for cardiac troponin T. The p value was calculated by Student's *t* test for log-transformed AUC. (B) Exposure to an oxygen-deprived medium results in a decreased contractile force developed by atrial trabeculae in isometric conditions. Replacement of the ischaemic buffer by an oxygen-enriched solution (mimicking reperfusion) allowed contractile function recovery. Means (SDs) are presented. p values are for the afternoon group *vs* morning group at the same reperfusion time and were calculated by post-hoc *t* test. (C) Volcano plot comparison of gene expression in atrial tissue between morning and afternoon biopsies, taken from 18 patients operated on in the morning matched with 11 patients operated on in the afternoon. The x-axis indicates the fold change ratios in a log2 scale, the y-axis indicates the statistical significance of the fold change (false discovery rate-corrected) on a log10 scale. Significantly dysregulated genes (fold change>1·2, p<0·05) are represented by red (upregulated) or green (downregulated) squares, and genes belonging to the circadian rhythm gene ontology category are indicated by an arrow. (D) Bar graph showing differentially expressed genes with relative individual expression levels displayed on a linear scale. *p<0·005 *vs* afternoon. (E) Gene expression in human atrial tissue measured by real-time quantitative PCR (n=28–30 samples per group). Data are means (SDs) of expression levels relative to those in the morning atrial tissue arbitrarily set to 1. p values are for the expression level in the afternoon group *vs* the morning group.

assigned to undergo surgery in the morning (n=44) or the afternoon (n=44; appendix p 16). The randomised study ended because of inclusion of the prespecified 44 patients per group, at a median follow-up of 12 days until hospital discharge (IQR 9–16). We did not observe any unintended effects or harms in each group related to the procedure, and the two groups were well matched, with similar intraoperative characteristics (table 3). However, the postoperative geometric mean cardiac troponin T AUC was significantly lower in the afternoon group (179 ng/L [95% CI 161–198]) than in the morning group (225 ng/L [199–255]), with an estimated



	Morning surgery (n=44)	Afternoon surgery (n=44)	OR (95% CI) for time-of-day effect pm *vs* am	p value
Death during hospital stay	0	0	NA	..
Need for inotropic support	4 (9%)	1 (2%)	0·25 (0·03–2·17)	0·20
Postoperative atrial fibrillation	16 (36%)	12 (27%)	0·75 (0·40–1·39)	0·36
Perioperative myocardial infarction	7 (16%)	2 (4%)	0·29 (0·06–1·30)	0·11
Cardiac troponin T AUC (ng/L×72 h)	225 (199–255)	179 (161–198)	0·79 (0·68–0·93)	0·0045*
Left ventricular ejection fraction <45% at discharge	5 (11%)	2 (4%)	0·40 (0·08–1·96)	0·25
Duration of hospital stay, days	12 (3)	12 (4)	0·99 (0·89–1·12)	0·89

Data are n (%), geometric mean and 95% CI for the mean (in the case of cardiac troponin T AUC), or mean (SD). OR=odds ratio. AUC=area under the curve. *p value for log-transformed (cardiac troponin T AUC).

Table 4: **Postoperative events until hospital discharge in the 88 randomised patients**

	Coefficient	Standard deviation	p value
Log (troponin AUC)			
Afternoon surgery	–0·1084	0·0338	0·0019
Aortic cross-clamping duration	0·0021	0·0009	0·0296
Log (troponin AUC)			
Afternoon surgery	–0·1179	0·0327	0·0005
Aortic cross-clamping duration	0·0018	0·0009	0·0476
Serum creatinine concentration	0·0232	0·0084	0·0073

The effect of the time of the day of surgery on perioperative myocardial injury—ie, log (troponin AUC)—was adjusted to aortic cross-clamping duration and serum creatinine concentration by a multivariable linear regression model with no variable selection. AUC=area under the curve.

Table 5: **Adjustment of the time-of-the-day effect on perioperative myocardial injury by multivariable analysis in the 88 randomised patients**

geometric means ratio for afternoon:morning of 0·79 (95% CI 0·68–0·93; p=0·0045; figure 2A, table 4). This time-of-the-day effect was also recorded upon analysis of data stratified for the two operating surgeons (appendix p 17) and remained significant after adjustment for aortic cross-clamping duration and serum creatinine concentration (table 5, appendix p 13).

Taken together, these data indicate that morning–afternoon variation in perioperative myocardial injury exists in patients undergoing aortic valve replacement, with afternoon surgery providing perioperative myocardial protection and better outcome.

We next explored whether these differences in morning and afternoon patients are related to intrinsic differences in myocardial ischaemic–reperfusion tolerance by subjecting human myocardial samples to hypoxia–reoxygenation conditions ex vivo. Atrial myocardial samples obtained freshly via biopsy from patients in the morning (n=14) and the afternoon (n=16) were studied. Contraction recovery after the hypoxia–reoxygenation challenge was significantly better in myocardial samples obtained from biopsies taken from patients randomised to afternoon surgery than those assigned to morning surgery, again despite no discernible differences in patient characteristics (figure 2B, appendix p 14).

To assess the mechanism responsible for the morning–afternoon variation in ischaemia–reperfusion tolerance, we did a transcriptomic analysis on morning and afternoon human myocardium biopsy samples. The analysis showed that the expression of 287 genes was regulated by the time of the day; these genes showed statistically significant differences in their relative expression between the morning and the afternoon (figure 2C). Biological term annotation of the differentially expressed gene list against the Kyoto Encyclopedia of Genes and Genomes database showed a highly significant enrichment of the biological theme "circadian rhythm", suggesting that this process may be involved in the time-of-the-day myocardial ischaemia–reperfusion tolerance in the patients (figure 2C, 2D). In line with the circadian regulation of myocardial gene expression including previous results on human left ventricular myocardium,[23] the nuclear receptor and transcriptional repressor Rev-Erbα and its target gene *BMAL1/ARNTL* displayed anti-phasic time-of-the-day expression variation, with *BMAL1/ARNTL* being prominently expressed during the afternoon when Rev-Erbα levels are low (figure 2E). Thus, the human myocardium displays an intrinsic morning–afternoon variation in hypoxia–reoxygenation tolerance concomitantly with transcriptional alterations in circadian gene expression.

Since *Rev-Erbα* was one of the most dynamically regulated genes between the morning versus afternoon (figure 2D, 2E) and a relevant pharmacological target with available synthetic ligands,[22] we assessed whether targeting the Rev-Erbα signalling pathway might change the differences in myocardial hypoxia–reoxygenation tolerance between the morning and afternoon by inhibiting Rev-Erbα activity, either by *Rev-Erbα* gene ablation (knockout) or treatment with its synthetic antagonist SR8278, in isolated mouse hearts (see appendix pp 5–7, 15, 18–24 for detailed results and figures). Both *Rev-Erbα* gene deletion and pharmacological inhibition conferred myocardial hypoxia–reoxygenation tolerance at the sleep-to-wake transition, acting via its downstream effector CDKN1a/p21 (appendix pp 5–7).

Discussion

The results of our study show a clinically significant morning versus afternoon variation in myocardial tolerance to the controlled ischaemia–reperfusion insult imposed during cardiac surgery, with patients undergoing aortic valve replacement in the afternoon displaying a lower perioperative myocardial injury and postoperative morbidity than those operated on in the morning. Unbiased transcriptome analysis of cardiac biopsies identified circadian genes with expression of the pharmacological target Rev-Erbα being highest in the

morning. In mice, *Rev-Erbα* gene deletion or antagonist treatment prevented the hypoxia–reoxygenation injury at the time of sleep-to-wake transition in an ex-vivo Langendorff model of hypoxia–reoxygenation myocardial injury, through a mechanism involving altered expression of the ischaemia–reperfusion modulator CDKN1a/p21.[24]

Despite major improvements in surgical and cardiac protection techniques, cardiac surgery with extra-corporeal circulation requires cardioplegia and exclusion of the heart from the general circulation by aortic cross-clamping—ie, provoking a sequence of myocardial ischaemia–reperfusion.[1–3] This problem remains a major issue even though trans-catheter valve implantation is increasingly used.

Mixed results have been reported regarding the existence of a biorhythm in myocardial ischaemia–reperfusion tolerance with clinical consequences in the context of STEMI.[11–15] From this inconsistency, several concerns have arisen. First, a so-called human factor, rather than a biorhythm in ischaemia–reperfusion tolerance, has been suspected.[25] Indeed, the reported worst outcomes for myocardial infarction occur during off-hours duty (ie, between the hours of midnight and 0600 h) for the medical staff, potentially because of reduced efficiency of the staff.[15,25] Second, geographical variation, latitude, seasonal factors, and ethnic differences could have been confounding factors in multicentre studies because both geographical and ethnic differences affect regulation of circadian genes.[26,27] We chose to study scheduled aortic valve replacement in a single-centre study to alleviate these concerns, and to show the existence of a biorhythm in cardiac ischaemia–reperfusion tolerance. Indeed, aortic valve replacement is a well-described surgical procedure with little variation between experienced surgeons such as the senior surgeons involved in our study. Accordingly, cardiopulmonary bypass and aortic cross-clamping durations were very similar between the morning and afternoon groups. Moreover, heart manipulation by the surgeon during aortic valve replacement is minimal, by contrast with that which occurs during coronary surgery. Thus, the main determinant of troponin release after aortic valve replacement is the duration of the aortic cross-clamping, rather than a human factor as has already been shown by others.[3] Hence, we found a significant effect of the time of the day of surgery on troponin release in the days following surgery, which most likely results from a biorhythm of ischaemia–reperfusion tolerance of the human myocardium in accordance with the results from our transcriptomic studies.

We postulated that the observed variation in ischaemia–reperfusion tolerance is caused by an intrinsic biorhythm in the cardiomyocyte. Therefore, to exclude nervous system, inflammatory, and coagulation processes, we studied both human and rodent myocardia. We consistently show in these two models that the myocardium displays an intrinsic biorhythm in ischaemia–reperfusion tolerance, extending findings from animal studies of myocardial infarction.[8–10] We further show that the morning–afternoon variation in ischaemia–reperfusion tolerance is paced in synchrony with core circadian machinery transcripts in the human myocardium. Down-regulation of Rev-Erbα signalling by gene inactivation or antagonist treatment decreased the elevated hypoxia–reoxygenation injury noted at the time of maximal Rev-Erbα expression levels. Transcriptomics identified CDKN1a/p21 as a downstream target of Rev-Erbα in the human myocardium. As has already been shown in the liver,[28] Rev-Erbα directly represses the transcription of CDKN1a/p21, which protects cardiomyocytes from cell death.[24] Overall, our data are consistent with a role of Rev- Erbα as a master switch in cardiomyocyte ischaemia–reperfusion tolerance.

We recorded lower postoperative cardiac troponin AUC together with a lower incidence of perioperative (type 5) myocardial infarction in patients operated on in the afternoon. The size of the afternoon effect on perioperative myocardial injury was very similar to what has already been demonstrated with other cardioprotective strategies, such as ciclosporin and remote ischaemic conditioning,[3,18] and its association with postoperative prognosis was consistent with published data.[4,18] However, although myocardial loss was intuitively related to postoperative heart failure, the decreased perioperative myocardial injury in the afternoon was probably not the only parameter responsible for the reduced incidence of subsequent heart failure in the afternoon group. Indeed, heart failure occurred months after aortic valve replacement despite preserved left ventricular ejection fraction at discharge in almost our entire studied population. Speculatively, in view of the interaction between circadian biorhythm and cardiac remodelling in pre-clinical models,[8] the biorhythm affecting myocardial tolerance to ischaemia–reperfusion might also affect postoperative left ventricular diastolic (dys)function, left ventricular reverse remodelling, subsequent atrial fibrillation, and renal function. Further studies specifically dedicated to understanding the mechanism behind the lower frequency of heart failure development after afternoon aortic valve replacement than after morning surgery are clearly warranted and should consider the interplay between circadian rhythms and systemic responses to surgery such as postoperative inflammation.

Although a single-centre study allows us to avoid many confounding factors resulting from heterogeneous perioperative patient management (eg, variations in anaesthetic drugs and cardioplegia), our findings do require validation in a multicentre study. In the cohort study, patients undergoing aortic valve replacement in the afternoon displayed lower rates of major adverse cardiac events than those operated on in the morning, but confident intervals were quite wide since the number of events was low, which is indicative of the current

high-quality patient management during and following aortic valve replacement.

The randomised study was designed to test the effect of time-of-the day surgery on perioperative myocardial injury, assessed by measurement of the troponin AUC, and was not powered to show an effect on clinical outcomes. Moreover, patients with diabetes and renal insufficiency were excluded to increase perioperative risk homogeneity. Further studies on these patients are therefore warranted, since they represent a population with high cardiovascular risk.

The animal studies, despite providing mechanistic insight and proof-of-concept of a pharmacological approach, require confirmation in human beings. Moreover, the mouse is a nocturnal species. Nevertheless, as has already been shown in human and rodent myocardium,[9,23] and confirmed in our study, the pattern of circadian gene expression is the same when considering the sleep-to-wake and wake-to-sleep transitions as reference.

Finally, the association between perioperative ischaemia–reperfusion injury and medium-term clinical outcomes remains correlative. Moreover, time-of-the-day probably interferes with different systemic processes and as such might affect other organs (eg, kidneys and immune cells).

Based on our findings, a large, prospective, multicentre, randomised trial, designed to investigate clinical outcomes in patients undergoing either morning or afternoon cardiac surgery, is warranted.

To put our findings into perspective, a few years ago, David J Lefer wrote an editorial asking "whether there is a better time to have a heart attack?"[29] We show here that the afternoon is probably a better time to undergo cardiac surgery than the morning, and is associated with a better mid-term prognosis. Importantly, cardioprotection in conditions of ischaemia–reperfusion injury concerns not only on-pump cardiac surgery, but also STEMI and cardiac transplantation. Although consideration of the time of cardiac surgery (eg, afternoon surgery) is an option, this is not possible for STEMI and organ transplantation because the time of STEMI onset and death of the organ donor are unpredictable. Therefore, the consequences and applications of identifying the cardioprotective potential of modulation of Rev-Erbα activity are far greater than the sole situation of on-pump cardiac surgery. *Rev-Erbα* is a unique circadian gene belonging simultaneously to the molecular circadian clock and to nuclear receptor families. As such, by contrast with most other circadian genes, it is a relevant pharmacological target, and several small molecules acting through this receptor have been identified.[22,30] We provide the proof-of-concept that Rev-Erbα antagonism is potentially a useful strategy to limit ischaemia–reperfusion injury. The design and synthesis of novel compounds with higher activity and refined pharmacokinetic profiles is thus warranted to develop clinically effective and safe drugs.

In conclusion, perioperative myocardial injury is transcriptionally orchestrated by the circadian clock in patients undergoing aortic valve replacement with Rev-Erbα antagonism emerging as a pharmacological strategy for cardioprotection. Consideration of the timing of surgery might also lead to improved outcome, with afternoon surgery providing perioperative myocardial protection and better patient outcomes.

Contributors

DM, XM, PL, and BS initiated the project, generated research funds and ideas, led and coordinated the project, interpreted data, and wrote the paper. DM and XM initiated and performed the in-vivo and ex-vivo experiments on human and mouse myocardia. AC, SM, CP, SN, and CK also contributed to the ex-vivo experiments. TM, GF, BJ, and MK did the cardiac surgery and human atrial biopsy. DM, AC, SM, CP, SO, CS, SN, and CK were responsible for patients' inclusion, follow-up, and adjudication of outcomes. AB, CG, and JE did the molecular biology and transcriptomic analyses. RD and BD did the pharmacokinetic studies. PL did the bioinformatics analyses. HD and DL provided intellectual input. DM and J-LE did the statistical analyses. All authors commented on the report, and have seen and approved the final version.

Declaration of interests

We declare no competing interests.

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