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Eplerenone does not limit ischemia–reperfusion injury in human myocardial tissue☆

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ABSTRACT

Background: Despite rapid reperfusion, mortality and morbidity in patients with an acute myocardial infarction remain significant. Therefore, novel pharmacological strategies to further limit ischemia–reperfusion (IR) injury are warranted. In animal models of myocardial infarction, mineralocorticoid receptor antagonists potently limit infarct size. In the current study we aimed to translate these findings to the human situation and investigated for the first time in human myocardial tissue whether eplerenone limits IR-injury.

Methods: In 24 patients undergoing elective cardiac surgery, the right atrial appendage was harvested, and two trabeculae were dissected from each appendage and suspended in an organ bath. We induced contraction by electrical field stimulation. Recovery of contractile force after a period of simulated ischemia and reperfusion was used as well-validated endpoint of IR-injury. From each patients, the trabeculae were randomized to either ischemic preconditioning (IP) or no IP ($n = 12$; positive control experiment) or to superfusion with eplerenone (10 μM) or vehicle ($n = 12$) in a paired approach.

Results: IP improved recovery from 19.9 (SEM 3.3)% to 26.3 (SEM 4.3)% ($p < 0.05$). During vehicle and eplerenone superfusion, mean recovery of contractile function after simulated ischemia and reperfusion was 45.2 (SEM 5.6)% and 36.5 (SEM 4.1)% ($p = 0.14$).

Conclusion: Eplerenone does not limit IR-injury in human atrial tissue ex vivo. Our results are in sharp contrast to preclinical studies demonstrating cardioprotective effects of mineralocorticoid receptor antagonist. With great interest we await the results of the MINIMISE-STEMI study, in which the effect of MR antagonism on myocardial infarct size in humans is currently under investigation.

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1. Introduction

Rapid myocardial reperfusion is essential to limit infarct size in patients with a myocardial infarction. Paradoxically, reperfusion itself can also aggravate injury ("reperfusion injury") [1]. Therefore, mortality and morbidity of these patients remain high, and novel strategies to reduce ischemia–reperfusion (IR) injury are needed. It has been suggested that mineralocorticoid receptor (MR) antagonists might serve this goal, since these drugs reduce morbidity and mortality in patients with heart failure [2–4]. Indeed, direct cardioprotective effects of these drugs are consistently demonstrated in several murine models of myocardial infarction [5–10]. The acute administration of MR antagonists, either before the onset of ischemia or at the moment of reperfusion, profoundly reduced infarct size (reviewed in [11]). In an elegant series of experiments it has been shown that the cardioprotective effects of the MR antagonists eplerenone and canrenoate depend on extracellular adenosine formation [9]. Adenosine is an endogenous purine nucleoside, and stimulation of membrane-bound adenosine receptors induces various effects, including attenuation of inflammation, vasodilation and protection against IR [12].

Whether these cardioprotective effects of MR antagonists also hold true in humans is yet unknown. In the current study, we aimed to translate the preclinical findings to the human situation for the first time and test the hypothesis that the MR antagonist eplerenone limits IR-injury in human myocardial tissue. We used the recovery of contractile function after a period of simulated IR in human atrial trabeculae as a well-established model of myocardial IR-injury [13–15].

☆ All authors take responsibility for all aspects of the reliability and freedom from bias of the data presented and their discussed interpretation.

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2. Methods

2.1. Patients

Adult patients undergoing elective coronary artery bypass surgery (CABG), valve surgery or aortic surgery, with extracorporeal circulation were asked to participate. Exclusion criteria were atrial arrhythmias, right ventricular failure, known atrial enlargement, the use of mineralocorticoid receptor antagonists, and the use of oral antiarrhythmics (except beta-blockers), sulfonylurea derivatives, dipyridamole, or theophylline. Patients were asked to abstain from caffeine consumption 24 h before surgery, since caffeine is an effective adenosine receptor antagonist which prevents the protective effects of ischemic preconditioning and might interfere with any protective effects of eplerenone [14]. All volunteers provided written informed consent before enrollment. The study protocol was approved by the Institutional Review Board of our center. The study was conducted in accordance with the Good Clinical Practices and the Declaration of Helsinki and was prospectively registered at ClinicalTrials.gov (NCT02118753).

2.2. Experimental design

We used the experimental set up as described previously [13,14]. Briefly, the right atrial appendage was harvested by the cardiothoracic surgeon before the introduction of the extracorporal circulation and immediately placed in cold (4 °C) modified Tyrode's solution (NaCl 118.5 mmol/l, KCl 4.8 mmol/l, $NaHCO_3$ 24.8 mmol/l, KH_2PO_4 1.2 mmol/l, $MgSO_4$ 1.4 mmol/l, $CaCl_2$ 1.8 mmol/l, glucose 10.0 mmol/l, and pyruvate 10.0 mmol/l), which was gassed with 95% oxygen and 5% CO_2. Two atrial trabeculae were dissected, vertically suspended in an organ bath, and linked to a force transducer. Each trabecula was superfused with pre-oxygenated Tyrode's buffer. Electrical field stimulation was performed in unstretched condition at 1 Hz using platinum ring electrodes placed on both sides of the trabeculae (pulse duration 60 ms; pulse current 40 mA). After 30 min of stimulation at unstretched conditions to allow recovery from transportation and preparation, trabeculae were gradually stretched over 15 min until maximal contractile force was achieved. After 20 min of equilibration, a baseline recording was performed during 10 min. Those trabeculae that failed to produce at least 0.2 g of developed force at the end of baseline were excluded. After 30 min of equilibration, the trabeculae were subjected to 90 min of simulated ischemia, followed by 105 min of reperfusion. Simulated ischemia was accomplished by superfusing the trabeculae with substrate-free modified Tyrode's solution (7.0 mM choline chloride substituted for glucose and pyruvate) and rapid pacing at 3 Hz. The superfusate was pumped into an artificial lung filled with 95% N_2/5% CO_2, which resulted in a low pO_2 of 10 to 20 mm Hg. For each patient, the trabeculae were randomized to either an intervention or control to allow paired analysis of the effects of the interventions.

First, as a positive control experiment, we studied the effect of ischemic preconditioning (IP) in 12 pairs of trabeculae, according to the experimental design we published previously [14]. Briefly, immediately after baseline recordings, the trabeculae of each patient ($n = 12$) were randomly assigned to either a stimulus for IP or continued superfusion with Tyrode's solution. IP was induced by 5 min of simulated ischemia and 5 min of simulated reperfusion.

Subsequently, the trabeculae of 12 patients were randomly assigned to superfusion with eplerenone (10 μM, kindly provided by Pfizer) or vehicle (dimethyl sulfoxide, DMSO < 0.1%). Administration of eplerenone started 10 min before simulated ischemia and continued throughout the experiment.

2.3. Data recording and statistical analysis

The differences between the maximal tension during contraction and the minimal tension during relaxation were averaged for every 5 min ('contractile force'). Recovery of contractile force in the last 10 min of reperfusion as a percentage of the last 10 min of equilibration was used as the primary endpoint.

For sample size calculation, we assumed a mean averaged percentage recovery of 18.7 ± 3.20%, and a test-to-test correlation coefficient of 0.5. A total of 12 subjects was required to detect a 37.5% change in averaged percentage recovery, which is a relevant reduction of ischemia–reperfusion injury, with a power of 80% at a significance level of 0.05.

Data are presented as mean (SEM) and were analyzed with SPSS 22 for Windows. We assumed a one-sided (IP) and two-sided (eplerenone versus vehicle) significance level of 0.05. Data were checked for normality and analyzed accordingly (Wilcoxon Rank test or Student's *t*-test).

3. Results

Of the 83 patients who initially signed their informed consent, successful paired experiments in two trabeculae were performed in 24 patients. The reasons for exclusion of the patients, and the reasons for drop-out are depicted in Fig. 1. The baseline characteristics of the patients are presented in Table 1.

In the positive control experiment, IP improved recovery from 19.9 (SEM 3.3)% to 26.3 (SEM4.3)% ($p = 0.048$), corroborating our previous finding [6]. In the second set of experiments mean recovery of contractile function after reperfusion was 45.2 (SEM 5.6)% and 36.5 (SEM 4.1)%, during vehicle and eplerenone superfusion, respectively ($p = 0.14$; Wilcoxon Rank test). After exclusion of one outlier with a recovery of 98.6% in the control trabecula, the results remained unchanged (40.4 (SEM 3.1)% and 36.0 (SEM 4.4)% for vehicle and eplerenone respectively; Fig. 2; $p = 0.17$ (Student's *t*-test)).

4. Discussion

Our study is the first to investigate the direct effects of mineralocorticoid receptor antagonism on ischemia–reperfusion injury in human cardiac tissue. In contrast to various recent preclinical studies, in which administration of MR antagonists consistently reduced myocardial infarct size in animal models of myocardial infarction [11], we did not observe any protective effect of eplerenone against IR-injury in human myocardial tissue.

Postischemic recovery of contractile force of human atrial trabeculae has been validated previously as a reliable and reproducible surrogate model of human myocardial IR-injury. Ischemic preconditioning consistently reduced IR-injury in this model in previous studies [13,14], which was reproduced by the first series of experiments in our current study. Moreover, the protective effect of IP in this model is critically dependent on adenosine receptor stimulation [14], opening of adenosine triphosphate-dependent potassium (K_{ATP}) channels, and activation of protein kinase C [13]. These mechanisms are of similar importance in IP-mediated cardioprotection in animal models of myocardial infarction using histological infarct size as endpoint of IR-injury [16]. These findings highlight that the mechanism of cardioprotection by IP is largely similar in animal models of myocardial infarction and the model we used in the current study.

In models of acute myocardial infarction in mice, rats, and rabbits, acute administration of spironolactone, eplerenone, or canrenoate profoundly limits infarct size [6–10]. Pathway analysis revealed that increased extracellular adenosine formation and subsequent stimulation of adenosine A_{2B} receptors is critical for eplerenone-induced cardioprotection [9]. Why do these consistent findings not translate to a cardioprotective effect of eplerenone in our current study?

First, it is known that in general the external validity of animal studies is limited due to biological differences between animals and humans. This is illustrated by the fact that about 500 neuroprotective treatment strategies improve outcome in animal models of ischemic stroke, while only two have proven effective in patients [17]. In addition, we used the recovery of contractile function as a marker for IR-injury.

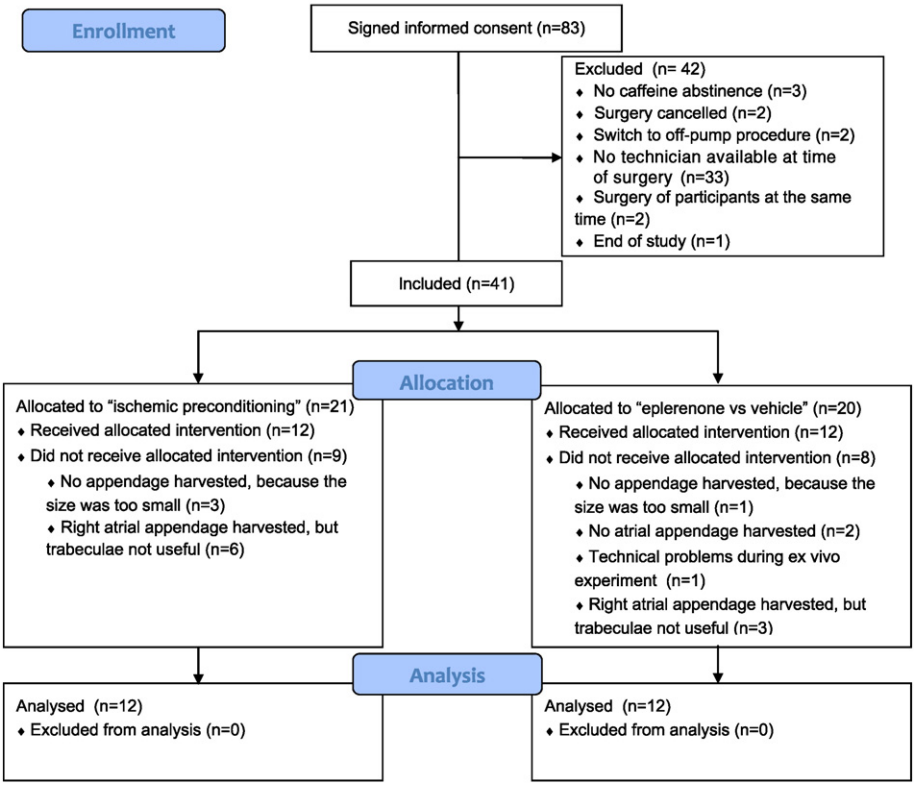

Fig. 1. Progress of participants during the experiment.

Table 1
Baseline characteristics.

Variable (n)	Setting 1 preconditioning (n = 12)	Setting 2 eplerenone vs control (n = 12)
Men	11	12
Age (mean ± SD)	55.4 ± 11.9	66.4 ± 8.1
Type of surgery		
CABG	6	8
AVR	4	2
CABG + AVR	0	1
Aortic surgery	2	1
Cardiovascular risk factors		
Hypertension	4	10
Diabetes mellitus	1	1
Current of former smoker	4	5
Positive family history	3	4
Dyslipidemia	7	4
BMI > 30 kg/m^2	2	1
Medication		
Aspirin	8	10
Beta blocker	10	9
ACE-inhibitor	4	5
ATII receptor antagonist	2	4
Ca-channel antagonist	1	5
Diuretic	1	5
Nitrate	1	1
Lipid lowering drug	9	4
Glucose lowering therapy	1	1

This differs from the studies in animals, in which histological infarct size is used as the primary endpoint. However, the protective effect of IP and pivotal involvement of adenosine, PKC, and K_{ATP}-channels that has been demonstrated in many animal models of myocardial infarction, could previously be confirmed in the atrial trabeculae model [13,14].

A second potential explanation relates to the presumed mechanism of eplerenone-induced cardioprotection. In animals, increased adenosine formation and receptor stimulation are critical for this effect. We have recently demonstrated, however, that eplerenone does not increase extracellular adenosine formation at relevant dosages in humans in vivo [18]. This finding is consistent with the lack of a cardioprotective effect in humans.

Thirdly, one could argue that the presence of the endogenous ligand aldosterone is required for the protective effect of MR antagonists. However, cardioprotective effects were also observed in hearts from adrenalectomized rats [8] and in Langendorff perfusion models [11], showing that the cardioprotective effect of MR antagonists in animals is independent from the presence of aldosterone.

Finally, it is important to consider the dose and timing of administration of eplerenone. The concentration of eplerenone that we used (10 μM), has been shown to limit IR-injury in a previous study [9] and is approximately 6 times higher than the calculated (peak) plasma levels in healthy adults after a one week treatment of eplerenone 50 mg bid [18]. Administration of eplerenone was started only 10 min before simulated ischemia. However, the limited duration of exposure to eplerenone does not explain its lack of benefit in the current study,

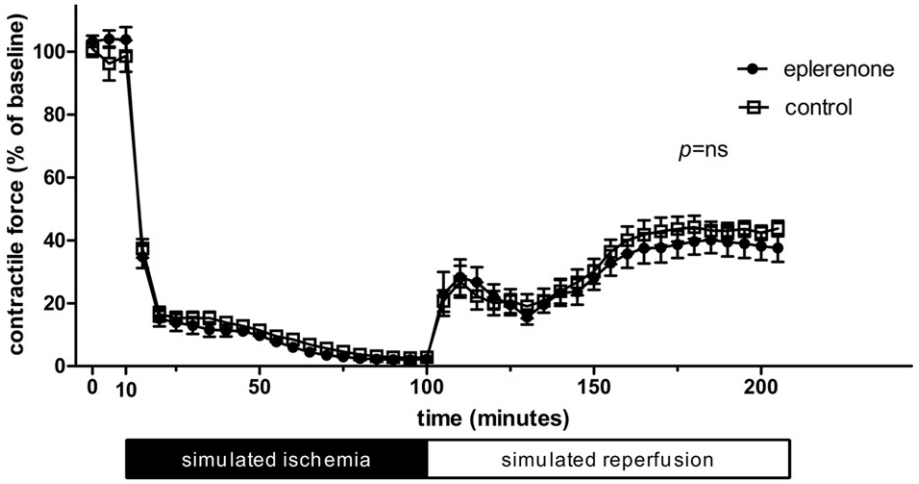

Fig. 2. Contractile force during eplerenone versus placebo. The contractile force (difference between maximal tension during contraction and minimal tension during relaxation) in paired trabeculae (n = 11) during eplerenone exposure (10 μM) (filled circles) or control (open squares); exclusion of 1 outlier.

since also in the animal studies, acute administration of MR antagonists effectively reduced infarct size [11].

In summary, eplerenone does not limit IR-injury in human myocardial tissue. These results are in sharp contrast to previous observations in animal models of IR. Based on these promising preclinical data, a large clinical trial was recently initiated to investigate whether MR antagonist limit infarct size in patients with an acute myocardial infarction [19]. Therefore, patients with a STEMI will be randomized to an intravenous bolus of canrenoate before coronary reperfusion, followed by three months of oral spironolactone. With great interest we await the results of this randomized controlled trial [19].

Author contributions

Conception and design of the study: TNAvdB, HAvS, JD, WM, GAR, NPR.

Acquisition of data: TNAvdB, JCV, VV, ACW.

Analysis and interpretation of data: TNAvdB, NPR, GR.

Drafting the article or revising it critically for important intellectual content: all authors.

Final approval of the version to be submitted: all authors.

Conflicts of interest

The authors report no relationships that could be construed as a conflict of interest. Financial support was obtained from the Netherlands Foundation for Cardiovascular Excellence (NFCVE). NP Riksen is supported by a grant from the Netherlands Heart Foundation (2012T051). NP Riksen received a non-restricted grant from AstraZeneca and has served on a Scientific Advisory Board of AstraZeneca unrelated to the subject of this paper.

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