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

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A B S T R A C T
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 patient, 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].
In a low pO2 of 10 to 20 mm Hg. For each patient, the trabeculae were disected, vertically suspended in 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% CO2. 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 trabecula 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 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 published previously [13,14]. Briefly, immediately after baseline recordings, the trabecula 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 trabecula 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 absolute change 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 ± 3.6% to 26.3 ± 7.8% (p = 0.048), corroborating our previous finding [6]. In the second set of experiments mean recovery of contractile function after reperfusion was 45.2 ± 8.5% and 41.6 ± 7.5%, 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 ± 10.5% versus 36.0 ± 10.4% for vehicle and eplerenone respectively; Fig. 2; p = 0.17 (Student’s t-test)).
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 KATP-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.

**Table 1**

Baseline characteristics.

<table>
<thead>
<tr>
<th>Variable (n)</th>
<th>Setting 1 preconditioning</th>
<th>Setting 2 eplerenone vs control</th>
</tr>
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<tbody>
<tr>
<td>Men</td>
<td>11</td>
<td>12</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>55.4 ± 11.9</td>
<td>66.4 ± 8.1</td>
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<tr>
<td>Type of surgery</td>
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</tr>
<tr>
<td></td>
<td>AVR</td>
<td>4</td>
</tr>
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<td></td>
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<tr>
<td></td>
<td>Aortic surgery</td>
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</tr>
<tr>
<td>Cardiovascular risk factors</td>
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</tr>
<tr>
<td></td>
<td>Current of former smoker</td>
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</tr>
<tr>
<td></td>
<td>Positive family history</td>
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</tr>
<tr>
<td></td>
<td>Dyslipidemia</td>
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</tr>
<tr>
<td></td>
<td>BMI &gt; 30 kg/m²</td>
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</tr>
<tr>
<td>Medication</td>
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</tr>
<tr>
<td></td>
<td>Beta blocker</td>
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</tr>
<tr>
<td></td>
<td>ACE-inhibitor</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>AT1 receptor antagonist</td>
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</tr>
<tr>
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<td>Ca-channel antagonist</td>
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<td></td>
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<tr>
<td></td>
<td>Nitrate</td>
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<td>Lipid lowering drug</td>
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</tr>
<tr>
<td></td>
<td>Glucose lowering therapy</td>
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</tr>
</tbody>
</table>

Fig. 1. Progress of participants during the experiment.
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

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References


