The effect of eplerenone on ischemia reperfusion injury in human myocardium

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Ethical review	Approved WMO
Status	Recruitment stopped
Health condition type	Coronary artery disorders
Study type	Interventional

Summary

ID

NL-OMON40313

Source
ToetsingOnline

Brief title
Eplerenone and ischemia-reperfusion injury

Condition

- Coronary artery disorders

Synonym
ischemia reperfusion injury, myocardial infarction

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Sint Radboud
Source(s) of monetary or material Support: Ministerie van OC&W

Intervention

Keyword: eplerenone, ischemia reperfusion injury

Outcome measures

Primary outcome

The effect of eplerenone of the recovery of contractile function after

simulated ischemia and reperfusion

Secondary outcome

none

Study description

Background summary

Coronary heart disease is the leading cause of death worldwide. Rapid myocardial reperfusion is the most effective strategy to limit infarct size in patients with a myocardial infarction. Paradoxically, the process of reperfusion of ischemic myocardium itself can also aggravate injury (*reperfusion injury*). Therefore, mortality and morbidity of patients with a myocardial infarction remain high, and novel strategies to reduce ischemia-reperfusion (IR) injury are urgently needed.

It has been suggested that the mineralocorticoid receptor (MR) antagonists spironolactone and eplerenone could potentially serve this goal, since treatment with these drugs reduces cardiovascular mortality in patients with heart failure (1). This hypothesis was recently confirmed in murine models of myocardial infarction, which demonstrated that the administration of MR antagonists, either immediately before the onset of ischemia, or at the moment of reperfusion, profoundly reduced infarct size (2,3). Interestingly, this cardioprotective effect was also observed in hearts from adrenalectomized rats (2), suggesting that this effect is not merely due to competitive antagonism of endogenous aldosterone, but could also be because spironolactone can function as an inverse agonist of the MR.

The underlying mechanism of the cardioprotective effect is currently unknown, but a recent study suggests the involvement of adenosine receptor stimulation (3). Adenosine is an endogenous purine nucleoside, which is known to increase the tolerance of various tissues, including the myocardium and blood vessels,

against ischemia and reperfusion (4). The extracellular enzyme ecto-5*-nucleotidase (also named CD73) is the rate-limiting enzyme in the extracellular formation of adenosine from adenosine monophosphate (AMP). Interestingly, in a recent study in mice, the cardioprotective effect of MR antagonists was abolished after targeted gene deletion of CD73. This observation suggests that adenosine is a key mediator of this effect. Interestingly, it has recently been shown that statins limit infarct size also by activation of myocardial CD73. The cardioprotective effects of MR antagonists have so far only been studied in isolated cells and in a few murine models of myocardial infarction.

Study objective

In the current research proposal, we aim to translate these preclinical findings to the human situation and test the hypothesis that MR antagonists limits IR-injury in human myocardial tissue. Secondly, we will test the hypothesis that the cardioprotective effect is mediated by adenosine receptor stimulation. Elucidation of this underlying signaling cascade is crucial to guarantee optimal future use of these drugs in clinical practice: if the cardioprotective effect is indeed mediated by increased extracellular formation of adenosine, then concomitant use of the adenosine receptor antagonist caffeine or theophylline will abolish this effect, whereas the adenosine uptake blocked dipyridamole could potentiate this effect.

Study design

This study is an ex vivo study on human atrial tissue that is harvested during coronary bypass surgery.

In patients undergoing coronary artery bypass surgery, prior to connection to the extracorporal circulation, the right auricle is harvested by the cardiothoracic surgeon.

Immediately after excision, the auricle will be placed in a cold (4° C) modified Tyrode*s solution, that is continuously gassed with 95% O2 and 5% CO2, as described previously (6). Subsequently, two atrial trabeculae will be isolated, suspended in organ bath, and connected to a force transducer. Contraction will be induced by electrical field stimulation. Ischemia and reperfusion will be simulated by superfusing the trabeculae with substrate-free and hypoxic modified Tyrode*s solution and rapid pacing at 3 Hz. The superfusate will be pumped into an artificial lung filled with 95% N2/5% CO2 to obtain a low pO2. The recovery of contractile force after 90 minutes of simulated ischemia and 120 minutes of reperfusion with oxygenated buffer will be used as endpoint of ischemia reperfusion injury. Using this experimental design, we have previously shown that a brief period of ischemia immediately before the prolonged period of simulated ischemia augments recovery of contractile function during reperfusion, which is called *ischemic

preconditioning* (6).

Experiment 1:
In vitro two trabeculae (diameter <1 mm; length >3 mm) will be dissected from the atrial appendage, vertically suspended in an organ bath, linked to a force transducer, and superfused with pre-oxygenated Tyrode*s buffer (pO2 500 to 600 mm Hg). Electrical field stimulation will be applied to induce contraction.
After equilibration, a baseline recording is performed during 20 min. Those trabeculae that fail to produce at least 0.2 g of developed force at the end of baseline or in which the coefficient of variation of developed force exceeded 20% are excluded.
Immediately after baseline recordings, for each patient the 2 trabeculae are randomly assigned to either a stimulus for IP or continued superfusion with Tyrode*s solution, so that from each patient 1 trabecula is preconditioned and the other was not. Ischemic preconditioning is induced by 5 min of simulated ischemia and 5 min of simulated reperfusion, as previously described (6). We will also start with this experiment in the current research proposal as a positive control, to ensure that the model is still working properly and it is possible to limit IR injury in this setting. Simulated ischemia is 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 is pumped into an artificial lung filled with 95% N2/5% CO2, which results in a low pO2 of 10 to 20 mm Hg. Subsequently, both trabeculae are subjected to 90 min of simulated ischemia and 120 min of simulated reperfusion. The percentage recovery (compared to second baseline) of contractile force of the trabeculae at the end of reperfusion (last 10 minutes) will serve as the primary endpoint. Should we not be able to reproduce our previous results that ischemic preconditioning augments recovery of contractile function, than the experiment will be stopped.

Experiment 2:
In the next 10 patients, a similar ischemia-reperfusion experiment will be performed, but now the two trabeculae from each patient will be randomized to either pretreatment with eplerenone (dissolved in DMSO, final concentration < 0.01%) or DMSO (vehicle), which will be present in the organ bath throughout the experiment. Eplerenone will be administered in a final concentration of 10 µmol/l in the organ bath, as this has been shown to limit myocardial infarct size in a previous study (3). This concentration is a relevant concentration, since pharmacokinetic studies in humans have shown that the plasma concentration after oral administration of 100 mg of eplerenone is approximately 3-6 µmol/l (6). Eplerenone will be added after 10 minutes of baseline recording, which will continue for another 10 minutes to be able to observe a direct effect of eplerenone on contractile force.

Experiment 3:
If the results of experiment 2 show a cardioprotective effect of eplerenone, than experiment 3 will be performed to investigate whether this effect is

dependent on adenosine receptor stimulation. In this experiment the trabeculae will be exposed to either eplerenone of eplerenone together with the adenosine receptor antagonist caffeine. Caffeine will be administered in a final concentration of 10 mg/l, which has previously been shown to significantly block ischemic preconditioning in a similar experimental design (7).

Experiment 4: if eplerenone does not show a cardioprotective effect in experiment 2, we will perform two additional studies:
4a: in this experiment, the trabeculae of 10 patients will be randomized to either pretreatment with aldosterone or vehicle to study whether aldosterone itself increases ischemia-reperfusion injury. Aldosterone will be administered in a final concentration of 10 nmol/l (which is slightly higher than the plasma aldosterone concentration in patients with primary hyperaldosteronism).
In addition experiment 4b will be performed in which experiment 2 will be repeated in the presence of 0.5 ng/ml aldosterone, which is similar to the normal circulating plasma concentration of aldosterone in humans.

Intervention

The intervention occurs ex vivo in the organ bath: adminstration of eplerenone with or without the adenosine receptor antagonist caffeine.

Study burden and risks

The removal of the right atrial appendage does not confer any risk to the patients.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

- age >18 years
- cardiac surgery with extracorporeal circulation

Exclusion criteria

* Use of theophylline
* Use of sulfonylureas
* Use of oral antiarrhythmics (not betablockers)
* Atrial arrhythmias
* Right ventricular failure
* Known atrial enlargement

Study design

Design

Study type:	Interventional
Intervention model:	Parallel
Allocation:	Randomized controlled trial
Masking:	Open (masking not used)
Control:	Placebo
Primary purpose:	Treatment

Recruitment

NL

Recruitment status:	Recruitment stopped
Start date (anticipated):	11-03-2014
Enrollment:	60
Type:	Actual

Ethics review

Approved WMO

Date:	23-01-2014
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL46830.091.13