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THE ROLE OF ALDOSTERONE INHIBITORS ON CARDIAC ISCHEMIA/REPERFUSION INJURY

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Abbreviations

11βHSD2 - 11β -hydroxisteroid dehydrogenase type 2

ACE- angiotensin converting enzyme

AF- atrial fibrillation

AMI- **acute** myocardial infarction

ATP- adenosine triphosphate

BH4- Tetrahydrobiopterin

BNP- brain natriuretic peptide

CNS- central nervous system

DBD - DNA-binding domain

DNA – deoxyribonucleic acid

FKBP51- FK506-binding protein 51

GR – glucocorticoid receptors

HF- heart failure

I/R – ischemia reperfusion

LAD - left anterior descending coronary artery occlusion

LBD – ligand

LV- left ventricle

MI- myocardial ischemia

mPTP- mitochondrial permeability transition pores

MR – mineralocorticoid receptor

NO- nitric oxide

NOS- nitric oxide synthase

NTD- N-terminal domain

NYHA- New York Heart Association

PKC- protein kinase C

PPI- proton pump inhibitors

RAAS – renin angiotensin aldosterone system

RNS- reactive nitrogen species

ROS- reactive oxygen species

STEMI- ST elevation myocardial infarction

TIMI- thrombolysis in myocardial infarction score

TIMP-1 - tissue metalloproteinase inhibitor-1

TTC- triphenyltetrazolium chloride

ABSTRACT

Myocardial ischaemia-reperfusion (I/R) injury is well known term for exacerbation of cellular destruction and dysfunction, after the restoration of blood flow to previously ischaemic heart. A vast number of studies that has demonstrated the role of mineralocorticoids in cardiovascular diseases is based on the use of pharmacological mineralocorticoid receptor (MR) antagonists. This review paper aimed to summarize current knowledge on the effects of MR antagonists on myocardial I/R injury, as well as post-infarction remodeling. Animal models, predominantly Langendorff technique and left anterior descending coronary artery occlusion (LAD) have confirmed the potency of MR antagonists as preconditioning and postconditioning agents in limiting infarct size and postinfarction remodeling. Several preclinical studies in rodents have established and proved possible mechanisms of cardioprotection by MR antagonists, such as reduction of oxidative stress, reduction of inflammation and apoptosis, therefore limiting infarct zone. However, the results of some clinical trials are inconsistent since they reported no benefit of MR antagonists in acute myocardial infarction. Due to this, further studies and the results of on-going clinical trials regarding MR antagonists administration in patients with acute myocardial infarction are being awaited with great interest.

Key words: aldosterone inhibitors – ischemia/reperfusion – clinical models – animal models

INTRODUCTION

A vast number of studies that has demonstrated the role of mineralocorticoids in cardiovascular diseases is based on the use of pharmacological mineralocorticoid receptor (MR) antagonists (Weldon and Brown 2019). These antagonists have shown a beneficial effect in various animal models of disease as well as in several clinical trials and represent potential prevention therapy for patients at risk of ischemic disease. Currently, the most used MR antagonists in humans are spironolactone and eplerenone. Both have similar therapeutic benefit but different pharmacological characteristics (Weldon and Brown 2019; Namsolleck and Unger 2014). However, another novel MR antagonist finerenone attracted the attention of researchers due to its beneficial pharmacokinetic and pharmacodynamics properties.

Ischaemia-reperfusion (I/R) injury can be defined as the exacerbation of cellular destruction and dysfunction, after the restoration of blood flow to previously ischaemic tissues. Timely restoration of blood flow is crucial for salvation of ischemic tissues. However, reperfusion causes more damage (Kolkhof et al. 2016; Domenic 2015; Grombein et al. 2015). Up until now several animal models of myocardial I/R injury have been established with three predominantly used methods such as the Langendorff model of global ischemia, the isolated working heart model according to Neely, and the left anterior descending coronary artery (LAD) occlusion model.

Despite various reperfusion therapies, morbidity and mortality has remained high in patients with acute myocardial infarction (AMI). MR antagonists have become very attractive candidates for this purpose, as preconditioning agents, since numerous clinical trials in patients with heart failure have reported a survival benefit induced by MR antagonist treatment. Numerous preclinical animal studies have revealed possible mechanisms of cardioprotection via spironolactone and/or eplerenone, including alleviation of oxidative stress, reduction of inflammation, inhibition of apoptosis and reduction of collagen fibers. Nevertheless, exact mechanisms of cardioprotection by MR antagonists remained unclear, mostly due to inconsistent results of animal and human studies (Buonafine et al. 2018).

The aim of this review paper was to summarize current knowledge on the effects of MR antagonists on myocardial I/R injury, as well as post-infarction remodeling. In order to explain these effects, distribution and

function of the MR throughout the body and cardiovascular system and underlying molecular mechanisms of cardioprotection will be discussed thoroughly.

DISTRIBUTION AND FUNCTION OF THE MINERALOCORTICOID RECEPTORS

MR displays a modular structure comprising several separate domains with specific functions (Arriza et al. 1987). It belongs to the superfamily of nuclear receptors and it contains three major functional domains: an N-terminal domain (NTD), a central DNA-binding domain (DBD) and a C-terminal or ligand binding domain (LBD). The MR-NTD is considered to be intrinsically disordered enabling structural flexibility and diverse protein interactions. The MR-NTD contains an activation function (AF), AF-1, which location has been variously identified as AF1a, MD (middle domain), and AF1b with a central inhibitory region that may reflect the ability of the MD to recruit corepressors (Pascual-Le Tallec et al. 2004). The DBD contains two zinc ions tetrahedrally coordinating four cysteine residues with residues important for DNA recognition and binding, as well as for receptor homo- and hetero-dimerization(Hudson et al. 2014) .It is linked to the LBD by a hinge region of 61 amino acids that may also play a role in receptor dimerization (Savory et al. 2001). The MR in transcriptionally inactive state with no ligands binded, is tied to heat shock protein 90 (hsp90) and FK506-binding protein 51 (FKBP51) in the cytoplasm, which enables the maintenance of the inactive conformation of the MR. When the endogenous ligand (aldosterone or cortisol) binds to MR conformational change happens, which allows the recruitment of FKBP52 and subsequent nuclear transfer. Afterwards, inside the nucleus dissociation of Hsp90 allows MR dimerization and consequent transcription of the MR target gene (Fuller et al. 2019).

Primary endogenous ligand for MR is the mineralocorticoid hormone aldosterone which is synthesized from cholesterol in the adrenal cortex and is an important regulator of blood pressure and electrolyte balance (Nappi and Sieg 2011). Extra-adrenal sites of aldosterone synthesis have also been identified, including brain, vascular tissue and myocardium (Toda et al. 2013). Synthesis and release of aldosterone are stimulated by low sodium or high potassium levels in the plasma or by direct stimulation via angiotensin II (Barrera-Chimal et al. 2019). When aldosterone is secreted from the adrenal glands, it binds to the MR in the renal tubule cell and forms a complex

(Bousqueta et al. 2019). This complex enhances transcription of specific DNA segments in the nucleus, leading to the formation of two protein transporters, Na^+/K^+ ATPase pump and Na^+ channel. These protein transporters increase sodium reabsorption and potassium excretion in the distal tubule and the collecting duct of the kidneys, which helps the body to maintain normal volume status and electrolyte balance, increasing the blood pressure (Buonafine et al. 2018; Bousqueta et al. 2019).

That means that aldosterone promotes sodium and water retention while inducing potassium and hydrogen ion excretion via activation. Chronically elevated plasma aldosterone levels might be the cause certain cardiovascular diseases such as hypertension, congestive heart failure and reactive myocardial fibrosis and thus contribute to cardiac remodeling and its adverse consequences (Weldon and Brown 2019; Barrera-Chimal et al. 2019; Tam et al. 2017). Nevertheless, MR function is much wider, due to its distribution in several cell types and tissues in the body.

Distribution of MR throughout the body includes several tissues, both epithelial and non-epithelial tissues. Function of MR is being thoroughly investigated in the cardiovascular system, central nervous system, immune system, reproductive system and retina (Behar-Cohen and Zhao 2016), besides their primary location in the distal tubular cells of the kidney. When speaking about the expression of MR in cardiovascular system it should be mentioned that MR are co-expressed 11βHSD2 (11β -hydroxisteroid dehydrogenase type 2) in the smooth muscle cells and the endothelial cells of vasculature, while this co-expression is not present in the cardiomyocytes (Christy et al. 2003). It is already known for several decades that 11βHSD2 is an enzyme that converts cortisol to cortisone, making him unable to bind and activate MR but preferably aldosterone in the epithelial tissue. Additionally, wide MR expression in the central nervous system (CNS), especially hippocampus, has been the subject of extensive investigation since it is hypothesized that MR mediate diverse behavioral responses including memory and affect, but also stress and depression (de Kloet et al. 2016).

In order to completely understand the role of MR stimulation by aldosterone or by glucocorticoids it is necessary to know whether 11βHSD2 and perhaps 11βHSD1 are co-expressed with the MR in certain cell types. Studies show inconsistent results regarding this matter in the cardiovascular system. Namely, some authors revealed

the co-expression with 11βHSD2 in human cardiomyocytes and human vascular smooth muscle cells, rat vascular endothelial cells (Van der Berg et al. 2014), while the other ones claim the opposite that 11βHSD2 is absent in the cardiomyocytes(Christy et al. 2003; Smith and Krozowski 1996). In tissues where 11βHSD2is absent, such as the monocyte/macrophage cell types the interplay of activation of the MR and the glucocorticoid receptor (GR) is instructive. In other words, activation of the MR is proinflammatory, whereas the activation of the GR is classically antiinflammatory, with both responding to cortisol rather than aldosterone except in the conditions of pathological aldosterone excess (Rickard et al. 2009).

In the recent years, animal studies have shown that the over activation of MR may cause fluid accumulation in the retina, choroidal vasodilation, and promotion retinal neovascularization in hypoxic conditions. These results thus indicate the possible beneficial effect of the MR antagonists usage in retinal diseases treatment, such as central serous chorioretinopathy (Behar-Cohen and Zhao 2016). The presence of MR was also confirmed in the ovarium granulosa cells, where its role is in the regulation of the progesterone synthesis. Therefore, some scientists refer to progesterone as the additional third endogenous ligand of MR and natural antagonist of aldosterone (Caprio et al. 2008). Novel findings regarding the presence of MR in the adipose tissues opened a whole new spectrum of possible application of MR antagonists, including metabolic syndrome. In this location MR are thought to be the mediators of adipose differentiation, expansion and proinflammatory effects (Marzolla et al. 2012). All of aforementioned non epithelial locations of the functional MR opened a new era of research focusing on the complexity and selectivity of MR function in the organism.

ALDOSTERONE INHIBITORS - STRUCTURE AND MECHANISM OF ACTION

A vast number of studies that has demonstrated the role of MR in cardiovascular diseases is based on the use of pharmacological MR antagonists (Weldon and Brown 2019). MR antagonists decrease the aldosterone effect by binding to the mineralocorticoid receptor and therefore inhibiting aldosterone biological activity. This leads to higher levels of potassium in serum and increased sodium excretion, resulting in decreased body fluid and lower blood pressure (Nordqvist and Granberg 2019). These class of drugs has shown a beneficial effect in various animal

models of disease as well as in several clinical trials and represent potential prevention therapy for patients at risk of ischemic stroke (Weldon and Brown 2019; Namsolleck and Unger 2014).

MR antagonists can be divided into steroidal and non-steroidal compounds, according to their chemical structure (Buonafine et al. 2018). Obligate effect of MR blockade by steroidal MR antagonists was hyperkalemia and that was one of the triggers for additional activities on synthesis novel non-steroidal MR antagonists with novel physicochemical properties potentially reducing the risk hyperkalemia and possible arrhythmia. Spironolactone, eplerenone, canrenone have a steroidal structure while non-steroidal compounds are finerenone, apararenone, esaxerenone (Kolkhof et al. 2017). Two generations of MR antagonists are commercially available: spironolactone and canrenone belong to the first generation while eplerenone belongs to the second generation. The chemical structure of a much more specific second generation of MR antagonist is mostly based on the initial 17-spirolactone construct (Kim KE. 1996). Currently, the most used MR antagonist in humans are spironolactone and eplerenone. Both have similar therapeutic benefit but different pharmacological characteristics (Weldon and Brown 2019).

Spironolactone, a synthetic steroidal analog, was the first MR antagonist developed in 1959, about 30 years before the molecular characterization of the MR. Spironolactone is a γ- lactone of propionic acid. The starting compound in the synthesis was androstenedione, which via an intermediate, gives a compound with a lactone function, which contains a carboxyl group. By decarboxylation process, a compound that produces spironolactone by introducing a thioacetate group at position 7 is formed (H. Chen et al. 2004). The antagonist activity of the spirolactones has been linked to the presence of ά-lactone substituent at the steroid C17 position, which characterizes all spirolactone molecules. Another characteristic of spirolactones is the presence of a C7 side chain, which seems to modulate their antagonist efficiency (Corvol et al. 1978).

Due to the MR antagonism in the kidney, spironolactone results in increased excretion of sodium and water to lower fluid retention and lessen pressure on the heart (Gabbard et al. 2020). Spironolactone was first approved as a diuretic drug for the management of hypertension and primary aldosteronism, and later for edematous conditions in patients diagnosed with congestive heart failure. Also, spironolactone is indicated for hepatic cirrhosis and nephrotic syndrome, hypokalemia and severe HF (NYHA class III–IV) and when alternative treatments are inadequate or not tolerated (Seferovic et al. 2015; Lainscak et al. 2015). Antagonistic effect of spironolactone on MR is a very strong.

The effect is relatively nonspecific because it also binds to other steroid receptors, such as progesterone and androgen receptors, and causes endocrinal side effects such as gynecomastia and galactorrhea (Weldon and Brown 2019). Spironolactone has several active metabolites, including 7a-thiomethylspironolactone and canrenone. These metabolites confer a longer half-life of 13–17 hours and once-daily dosing efficacy of spironolactone (Yang et al. 2006).

Canrenone, is one of several active metabolites both of spironolactone and potassium canrenoate, which directly suppresses aldosterone and increase aqueous and sodium diuresis. In that manner, canrenone maintains potassium balance and acts as a diuretic (reducing preload and atrial volume overload). Canrenone prevents the genesis of intermediate products with anti-androgenic and progestational effects and thus canrenone induces less adverse events such as gynecomastia compare to spironolactone (Mantero et al. 2000). At variance with the spironolactone, canrenone does not require hepatic activation, thus minimizing potential confounders due to interindividual differences of liver function. Canrenone is the only RAAS-inhibiting agent that is clinically available as a solution for parenteral administration (Ramsay et al. 1976; Kim 1996). Canrenone plasma concentrations are five times higher after potassium canrenoate administration than the same dose of spironolactone. These results can explain with difference in their metabolism because a number of important sulfur-containing metabolites are not generated after potassium canrenoate administration (Kolkhof et al. 2017). The half-live of canrenone in healthy volunteers is 16.5h and in patients with cirrhotic ascites, this half-live can be even prolonged up to 58 h (Kolkhof et al. 2017). Thus, the long half-live of spironolactone's active metabolite canrenone is the basis of its efficacy when applied once daily among patients with essential hypertension or heart failure (Kolkhof. et al. 2017).

Eplerenone is a newer MR antagonists that is chemically derived from spironolactone (Abarbanell et al. 2010). It has been specifically developed to bind selectively to the mineralocorticoid receptors in order to minimize binding to the progesterone and androgen receptors and compared to spironolactone, eplerenone has an improved selectivity which limits undesirable side effects such as gynecomastia and vaginal bleeding (Bolli et al. 2004; Herr J et al. 2015; Bell M et al. 2011). The starting compound in the synthesis of eplerenone is the commercially available 11ά -hydroxyl canrenone, and the synthesis of eplerenone was performed in seven phases. The key intermediates in the synthesis are compounds with ether and the hydroxyl group which is suitable for the formation of compounds with a

new lactone function. In the penultimate phase of the synthesis, a compound was obtained, which contains a 7ά-methoxycarbonyl group. The double bond C9 = C11 was used to obtain eplerenone which features a 9, 11ά -epoxide in the lactone ring to confer mineralocorticoid selectivity (H. Chen et al. 2004).

Eplerenone is a well-tolerated selective MR antagonists used for the treatment of arterial hypertension, post-myocardial infarction LV dysfunction and systolic HF associated with mild to moderate symptoms (Herr et al. 2015). However, eplerenone has a reduced potency (approximately 40-fold lower affinity for MR) (Weldon and Brown 2019; Abarbanell et al. 2010). Affinity of eplerenone for mineralocorticoid receptors compared to spironolactone is lower and eplerenone shows 2–3% of spironolactone affinity in vitro. However, eplerenone is less firmly bound to plasma proteins and therefore it has approximately 60% the potency of spironolactone, Due to this reduced plasma binding to proteins, the metabolism of eplerenone is much faster compared to spironolactone. Eplerenone has no active metabolites and consequently, eplerenone's half-life is only 4-6h in steady-state, requiring twice-daily dosing (Cook et al. 2003; Seferovic et al. 2015).

Mexrenone, 9–11ά-epoxyderivatives of spironolactone, is a mineralocorticoid receptor antagonist that belongs to the steroid group containing a C-17 spironolactone side chain. It is a diuretic that has been used for the treatment of edematous states such as congestive heart failure and for the treatment of essential hypertension. Mexrenone, with a 7R-carbomethoxy function, is a more potent analog than canrenone (Preisig et al. 2003). The introduction of the epoxy group has decreased binding for the androgen and progesterone receptors of between 10- and 500-fold. With the reduced affinity for the androgen and progesterone receptor, there was a 3- to 10-fold reduction of the antiandrogenic and progestagenic effect compared to spironolactone (de Gasparo et al. 1987).

Novel MR antagonist of a third generation finerenone is lately being recognized as a drug worth of scientific attention thanks to its differences in pharmacokinetic and pharmacodynamic properties compared to first (spironolactone) and second generation (eplerenone) of MR antagonists. Namely, as one of the dihydropiridine-based non-steroidal MR anatagonist, finerenone has dihydronaphthyridine structure and combines high selectivity of eplerenone towards MR and high potency of spironolactone, with no affinity for L-type Ca^{2+} channels (Kolkhof et al. 2017). Finerenone has several beneficial features that set it apart from previous MR antagonists, such as no active metabolites, unlike spironolactone, short half-time of elimination (2.2-2.8h), high percentage of plasma

protein binding 90% and most importantly it is equally distributed in the heart and kidney tissue. This enables the usage of finerenone even in patients with renal insufficiency, which is the limitation of spironolactone and eplerenone (Kolkhof and Borden 2012).

MECHANISMS INVOLVED IN MYOCARDIAL I/R INJURY

For several decades, scientists have been struggling with discovering and explaining the complex mechanisms involved in the onset and progression of myocardial I/R injury for the purpose of implementing new therapeutic strategies for myocardium preservation. Up until now, following mechanisms have been pointed out as the ones that mostly mediate I/R injury: increased production of reactive oxygen species (ROS); depletion of ATP; Ca^{2+} overload; mitochondrial dysfunction and mPTP (mitochondrial permeability transition pores) opening; dysfunction of the endothelium; inflammatory response (Xia et al. 2016; Kalogeris et al. 2012; Sanada et al. 2011; Kalogeris et al. 2016). All these factors together subsequently lead cellular injury and cardiomyocytes death.

Ischemia leads to directing of metabolism towards anaerobic glycolysis, due to the lack of oxygen, and a decrease in ATP availability, thereby inhibiting Na^{+}/ K^{+} ATPase and increasing the amount of calcium in the cytoplasm. Additionally, in this stage pH is lowered due to accumulation of lactates and hydrogen ions. In the reperfusion period, since huge amount of oxygen is being delivered to the previously hypoxic myocardium, ATP synthesis, mitochondrial respiratory function and pH level are restored into normal rapidly. Nevertheless, because of the increased intracellular Ca^{2+} levels, Ca^{2+} uptake in sarcoplasmatic reticulum is promoted, and ryanodine receptors cannot support this amount of Ca^{2+}, which leads to its release in the cell. This phenomenon is called Ca^{2+} overload and it is associated with hypercontracture of the myofibrils. Also, increased intracellular calcium is significant in ischemic-reperfusion injury of the myocardium as it activates calcium-dependent proteases that induce apoptosis (Li et al. 2016). Besides this, in the first minutes of reperfusion, there is an increased production of prooxidants such as ROS and reactive nitrogen species (RNS), which are considered to be key initiators of I/R injury. Several cell types produce ROS, mostly in the ischemic zone, such as injured cardiomyocytes, endothelial cells and neutrophils. Subsequently, entrance of neutrophils in the ischemic zone causes the release of different types on proinflammatory cytokines and mediators and aggravates the cellular damages by causing inflammation. Metabolic adaptations

associated with ischemia and altered mitochondrial homeostasis are thought to affect increased RNS production in reperfusion. High levels of Ca^{2+} inside the cells also can promote ROS generation via the activation of xanthine oxidase. ROS are considered to be one of the crucial elements of I/R injury pathogenesis since they damage the cells in several ways. Besides inflammation, ROS lead to DNA damage, endothelium damage and also mitochondrial damage. This refers to ROS being the cause of mPTP opening together with increased Ca^{2+}. Opening of mPTP affects the release of cytochrome C and ultimately leads to cell death (Kalogeris et al. 2016; Li et al. 2016; Wu et al. 2011).

Protective role of nitric oxide (NO) on endothelium and cardiovascular system in general is well known due its proven antioxidative and anti-inflammatory properties. NO is produced by nitric oxide synthases (NOS) with endothelial eNOS being the most important one in the cardiac I/R pathology. However, when the myocardium is in hypoxic state, NOS is being converted into NOS uncoupling and therefore generates ROS and additionally contributes to oxidative stress and cell damage in cardiac I/R injury. Tetrahydrobiopterin (BH4) is structural part of NOS which concentration plays an important role in NO production. In I/R conditions, the BH4 tissue levels decrease and leads to uncoupling of NOS and superoxide anion radical, which results in cardiomyocytes death (Kietadisorn R et al. 2012).

Finally, translation of these pathophysiological mechanisms into clinical practice remains a challenge for clinicians, and the discovery of new algorithms that could benefit the reduction of reperfusion injury remains a matter of debate in the scientific community.

CLINICAL AND ANIMAL MODELS OF CARDIAC ISCHEMIA/REPERFUSION INJURY

Over the past few decades, large and still growing number of experimental interventions (both pharmacological and nonpharmacological) have been reported to be protective for the myocardium in I/R conditions in experimental animals. Different animal models can provide thorough information about novel mechanisms of myocardial and vascular disease. These models should be observed as potential clinical therapies. However, of hundreds different

interventions that have been proved to be cardioprotective, none has been translated into clinical practice, besides early reperfusion. The translation rate from the preclinical animal studies to the clinical bedside has remained low, probably due to differences in physiology of animals and humans and molecular pathways (Bell M et al. 2011; Rossello et al. 2016). Up until now several animal models of myocardial I/R injury have been established with three predominantly used methods such as the Langendorff model of global ischemia, the isolated working heart model according to Neely, and the left anterior descending coronary artery (LAD) occlusion model.

Langendorff model was firstly described by Oscar Langendorff in 1895. and ever since is used as a robust model for studying heart physiology and different pathologies such as I/R injury, last 40 years. Langendorff model of the isolated heart model enables acute observation of the functional effects of ischemia reperfusion injury in real time, including the effects of variety of pharmacological interventions administered at both before, during or after I/R episode. Given the fact that brief periods of ischemia can precondition the heart against I/R injury, *in situ* aortic cannulation is performed in order to enable functional assessment of non-preconditioned myocardium. Krebs Henseleit buffer which content is similar to physiological, is retrogradely perfused directly into the aorta. A saline-filled balloon is then inserted into the left ventricle and attached to a pressure transducer, which enables time measurement of pressures from within the ventricle and calculation of multiple functional cardiodynamic parameters. Ischemic injury is simulated by the stop of perfusion (coronary flow) which is commonly referred as global ischemia phase, followed by reperfusion period (restoring coronary flow). The duration of both ischemia and reperfusion period can be modulated depending on the study protocol, to examine biochemical events at any point of time. Although the Langendorff isolated heart model is not the best model for the consideration of systemic events affecting ischemia and reperfusion, it is an excellent model for the examination of acute functional and biochemical events within the I/R injury as well as the effect of pharmacological or non-pharmacological cardiac pre- and postconditioning (Rossello et al. 2016; Shao et al. 2013; Virag and Lust 2011; Gao et al. 2010). Langendorff model of myocardial I/R injury has been widely used for the examination of preconditioning properties of many drugs including MR antagonists (Yoshino et al. 2014). A modified form of Langendorff technique was developed in order to overcome its inability to quantify left ventricular work by Neely et al. (Kolk et al. 2009). The isolated working heart model includes the delivery of Krebs Henseleit solution into the left atrium via a cannula attached to a

peristaltic pump. The LV ejects this volume against a constant pressure head created by a hydrostatic column connected to the aorta. By providing volume for the LV to eject, the work of the LV can be can calculated. Other researchers have revised and taken Neely's modification one step further, by using a four chamber working model where all four chambers of the heart are filled and eject. Nevertheless, the majority of experiments using the isolated working heart involve small animals such as rats and mice, due to short breeding cycles and cost effectiveness.

Coronary heart disease is best mimicked via permanent or temporary ligation of the left anterior descending artery (LAD) or left circumflex coronary artery (LCX). This LAD occlusion model provides information about the systemic influences on the heart after myocardial I/R or AMI. Occlusion of the LAD is performed via left thoracotomy. Firstly, LAD is exposed and afterwards a suture or occlusion device is used to either temporarily occlude or permanently ligate the LAD or a branch of the LAD. Additionally, transient LAD occlusion can also be achieved by using ''closed chest'' methods. One of this kind of closed chest models still involves a thoracotomy to implant an occlusive device but I/R injury is not induced until few days after surgical procedure, when the acute inflammatory response from surgery has resolved. Other different closed chest model utilizes catheter-based occlusion with no thoracotomy (Kumar et al. 2016; Heusch et al. 2017; Hausenloy et al. 2015; Heusch and Rassaf 2016).

Unlike the previously described methods, via the LAD occlusion model, *in vivo* cardiac function but also infarct size can be measured following injury. Two-dimensional echo and MRI are most commonly used for assessment of *in vivo* heart function, but to quantify infarct size, triphenyltetrazolium chloride (TTC) can be infused into the LAD distal to the occlusion and before explanation of the heart. LAD occlusion model can be used in any animal species, but most commonly used are small rodents, such as mice and rats. Reason for this is most likely the short breeding cycles, ability to make transgenic animals, but also cost-effectiveness. In recent times, focal point of research interest has become the inclusion of the the hibernating myocardium in the peri-infarction zone, but also new insights in the pathophysiology of repetitive occlusion and reperfusion episodes, with more complex pathophysiology compared with the model where one-time ligation is used (Fuller et al.2017; Mahajan et al. 2018).

Many factors affect the critical time window for adequate cardioprotection, such as species due to the fact that infarct development is faster in small rodents with a high heart rate than in larger mammals, heart rate even

within an individual animal, infarct development occurs faster at higher heart rate (influence of anesthesia and adrenergic activation) and residual blood flow given the fact that at some residual blood flow through the stenosis or from collaterals, a state of short-term hibernation can develop which slows the development of infarction. Therefore, systematic animal studies regarding previously mentioned window of cardioprotection, in interaction with confounding these factors are needed (Yarnell et al. 2001).

Given the fact that most of these animal models are not applicable in the clinical settings, open chest surgery is the only option for confirmation of preclinical results. Several studies proved infarct size reduction after remote ischemic postconditioning in patients with reperfused acute myocardial infarction (Hausenloy and Yellon 2013; Xia et al. 2016)

When investigating MR antagonists role in myocardial I/R injury, both in clinical and preclinical studies, afore mentioned Langendorff and LAD animal models have been used in preclinical studies (Mahajan et al. 2018; Yoshino et al. 2014), while in clinical settings options were limited to atrial in vitro model, which investigates postischemic recovery of contractility of human atrial trabeculae. This model has been recognized as a reliable and reproducible analogue model of human myocardial I/R injury (Van den Berg et al. 2016).

THE PHARMACOLOGICAL TREATMENT OF CARDIAC ISCHEMIA/REPERFUSION INJURY

Myocardial ischemia (MI) is one of the leading causes of morbidity and mortality worldwide (Neri et al. 2017). Ischemia is characterized by an absolute or relative decrease in the blood supply of tissue or organ due to blockage of blood vessels while the restoration of blood flow after ischemia represents reperfusion. Timely restoration of blood flow to an ischemic heart is essential for limiting the infarct size but it can paradoxically exacerbate tissue damage. This phenomenon is called ischemia/reperfusion (I/R) injury and occurs in several forms such as reperfusion-induced arrhythmias, microvascular obstruction, myocardial stunning, and reperfusion-induced cardiomyocyte death (Kolkhof et al. 2016; Domenic et al. 2015; Grombein et al. 2015). Various medical and surgical strategies have been developed over the years to minimize the profound and detrimental effects on myocardial metabolism and contractility as well as on myocyte viability due to acute myocardial infarction (AMI).

Approaches are different but most often include the use of thrombolytic agents, β-adrenergic receptor antagonists, angiotensin converting enzyme (ACE) inhibitors, the use of antioxidants, the use of transluminal coronary angioplasty, coronary artery bypass grafting. One of the rare interventions that is universally accepted and still causes great attention by the scientific world is certainly the phenomenon of preconditioning of the heart by ischemia. Preconditioning is the phenomenon that an organism becomes tolerant or resistant to great stress if it has previously been exposed to a milder form of the same stress (Przyklenk et al. 1998; Vinod et al. 2000).

Ischemic preconditioning by exposing the heart to brief episodes of ischemia and reperfusion prior to prolonged ischemia is a potent protective endogenous adaptive response of the heart to long-term ischemic injury. Because the ischemic preconditioning is hardly possible in clinical practice, the attention is focused on the non-pharmacological and pharmacological methods that would be used prior to coronary occlusion or global ischemia in order to reduce the size of myocardial infarction (Li et al. 2015; Piriou et al. 2002).

Piriou et al. have considered the use of anesthetics such as desflurane, sevoflurane, isoflurane, and halothane as cardioprotective in a pharmacological preconditioning model. This group of drugs is thought that after it exerts its primary effect as anesthetics, to have a prolonged effect on the myocardium, which is interpreted as cardioprotective (Riess et al. 2004; Riess et al. 2004; Nagashima et al. 1999). There are also studies indicating that the use of pharmacological agents for preconditioning and, in addition to thrombolytic therapy, may reduce infarct size and incidence of tachyarrhythmias as well as the number of ischemic attacks in myocardium with unstable angina (Beisvag et al. 2003; Grimm et al. 1973).

Morphology of the rat heart is very similar to a human one. Structure of the left ventricular, properties of the papillary muscles and wall thickness are almost the same as in the human heart and thus a model of isolated rat heart is very reliable for connection between animal and human studies (Grimm et al. 1973). The study which examined the effects of the acute administration of calcium and potassium channel modulators on I/R injury in the isolated rat heart showed that all examined drugs have beneficial cardioprotective properties after ischemia and reperfusion. Administration of all agents was associated with lower values of major pro-oxidative molecules indicating that accomplished effects could at least in part be consequential in their antioxidative properties (Simonovic et al. 2018). Calcium channel blockers exert cardioprotective probably by inhibiting the action of slow

Ca^{2+} inward current through the L-type Ca^{2+} channels in the cardiac cells. Verapamil reduces oxygen demand by reducing preload, afterload, and contractility; improves oxygen supply to the ischemic zone; preserves the mitochondrial structure and function; and reduces the availability of calcium to stimulate ATPase, protease, and lipase (Yui et al. 2011; Yu et al. 2010). Yu and his team showed that when verapamil is administered for 10 minutes at a dose of 20 μmol / L, it leads to a significant improvement in diastolic and systolic function and reduces the incidence of arrhythmias occurring during the reperfusion period. This group of authors considers that one of the possible mechanisms for this effect is the reduction of Ca^{2+} influx, which stabilizes cardiomyocytes during ischemia and avoids the accumulation of Ca^{2+} that is released during I / R (Yu et al. 2010). Also, the findings of Okuda et al. highlighted that preconditioning with diltiazem at a dose of 10 mg / kg leads to a reduction in surface area coronary artery affected by infarction (Okuda et al. 1996). In a study focused at the acute effects of diltiazem on the isolated heart of rats on the Langendorff apparatus, which was administered immediately before or during global ischemia, this agent also has been shown to reduce the catabolism of adenine nucleotides, reduced myocardial oxygen demand and thus improved heart function (De Jong et al. 1984). Nicorandil, as a mitoCATP channel activator and nitric oxide donor, improves the recovery of contractile function of the heart after ischemia (Kitakaze et al. 2007; Ishii et al. 2005). Preconditioning with nicorandil affects the potential of the mitochondrial membrane and does not allow the development of mitochondrial dysfunction that would directly contributed to the emergence and development of remodeling and reduction of cardiac contractility (Wang et al. 2018).

The proton pump inhibitors (PPI) had also shown protective effects in the treatment of myocardial ischemia in patients with coronary artery disease and gastroesophageal reflux. The main mechanism of action of these drugs is the suppression of acid secretion by binding to H^+/K^+ ATPase known as "proton pump" (Monzani et al. 2010; Lindberg et al.1986). Proton pump exists in myocardial tissue and mechanical and electrical properties of the myocardium can be changed by using PPI (Nagashima et al. 1994). Omeprazole preconditioning in dose 100μM, had a protective effect on myocardium function recovery on the isolated rat heart and can be seen as an adjuvant type of myocardial protection primarily in terms of preserving the impact volume of that ejection fraction (Jeremic et al. 2015). Gomes et al have shown that the application of omeprazole and pantoprazole before the induction of ischemia, and in relation to the preconditioning ischemia groups, leads to protective effects on myocardial function

(Gomes et al. 2010; Gomes et al. 2011;). The mechanism of cardioprotective effect refers to changes in the transmembrane H^+/K^+ gradient, which is the first indicator of cardiac ischemia, responsible for the repolarization expressed in the T-wave morphology of the electrocardiogram (Gomes et al. 2004). On the other hand, pantoprazole has a negative effect on myocardial contractility, but also that in addition to its negative inotropic effect, pantoprazole does not lead to changes in intracellular pH. Under the action of pantoprazole, the uptake of calcium into the sarcoplasmic reticulum is reduced and the influx of calcium ions is reduced, which results in reduced calcium content in the sarcoplasmic reticulum and an increase in the concentration of Ca^{2+} ions during diastole (Schillinger et al. 2007).

As well, experiments in models of myocardial infarction have been reported that MR antagonist administrated before the induction of ischemia, and also in a period of reperfusion shown cardioprotective effects on I/R injury (Schmitt et al. 2004).

ALDOSTERONE INHIBITORS AND CARDIAC ISCHEMIA REPERFUSION INJURY

Despite various reperfusion therapies, morbidity and mortality has remained high in patients with AMI. MR antagonists have become very attractive candidates for this purpose, as preconditioning agents, since numerous clinical trials in patients with heart failure have reported a survival benefit induced by MR antagonist treatment. Additionally, experiments in animal models of myocardial infarction have demonstrated that acute administration of MR antagonists limits infarct size (Van den Berg et al. 2014). Figure 1 schematically shows mechanisms of cardioprotective effects of MR antagonists.

Mineralocorticoide receptors expression has been reported in multiple tissues, beginning with distal tubular cells of the kidney, but what is most important, is their location in the cardiovascular system in the following cell types: cardiomyocytes, cardiac fibroblasts, vascular smooth muscle cells, endothelial cells, as well as mononuclear cells. Due to this, they have been thoroughly investigated as preconditioning agents in myocardial I/R injury pathology over the years (Fuller et al. 2012). Large and still growing number of studies, both preclinical and clinical investigated the theme.

MR expressed in cardiomyocytes, fibroblasts, vascular endothelial, and smooth muscle cells mediate pathophysiological myocardial remodeling and fibrosis. Thus, the blockade of the MR and aldosterone actions provides clinical benefit in patients with heart failure. However, several limitations of MR antagonist usage have been elucidated, which is why they are not so frequently prescribed in clinical practice in heart failure patients. This refers to hyperkalemia especially when give in combination with RAAS blockers or potassium rich foods and risks in patients with kidney diseases. As we mentioned previously, structure dependent adverse effects such as gynecomastia, galactorrhea linked to spironolactone can be exceeded by non-steroidal MR. Regarding this, enthusiasm among scientist is growing with the aim of supporting novel MR antagonist finerenone investigation in heart failure and implanting this drug in clinical studies on humans.

1. Preclinical data

MR antagonists have become very attractive candidates for this purpose, as preconditioning agents, since numerous clinical trials in patients with heart failure have reported a survival benefit with MR antagonist treatment. Additionally, experiments in animal models of myocardial infarction have demonstrated that acute administration of MR antagonists limits infarct size. Nevertheless, exact mechanisms of cardioprotection by MR antagonists remained unclear, mostly due to inconsistent results of animal and human studies (Mihailidou et al. 2009). Table 1 summarizes data of interest from preclinical studies.

Several studies focused on the beneficial effects of spironolactone in acute myocardial infarction (AMI) and mechanisms involved in this. Earlier investigations suggest aggravation of cardiac damage in I/R conditions via aldosterone or cortisol perfusion through the heart by using Langendorff technique. This refers to increase in infarct zone and apoptosis of cardiomyocytes, after 30 min of ischemia followed by 2.5 hours of reperfusion period. These detrimental cardiac effects were reversed by MR inverse agonist spironolactone acting as a MR inverse agonist at a low dose of 10nM (Minicucci et al. 2013). In line with these results were also the findings of Marcos et al, who highlighted the spironolactone induced attenuation of cardiac remodeling after AMI in an animal model. Current investigation demonstrated that spironolactone reduced LV myocardium hypertrophy with no alterations in blood

pressure, ventricular enlargement, cardiac geometry, or diastolic and systolic ventricular function. This effect was explained via reduction of collagen type I and type III, which may be mediated through myocardial periostin reduction and tissue metalloproteinase inhibitor-1 in myocardium (TIMP-1) increase in the group of animals with AMI treated with spironolactone (Matsui et al. 2010). Periostin is matricellular protein of great importance which plays a crucial role in the maturation and differentiation of fibroblasts in the developing neonatal heart. In the AMI conditions, during the cardiac repair phase, periostin is released into the infarct zone and activates signaling pathways that are of great importance for the reparative process (Loan Le et al. 2012). Novel mechanisms of cardioprotection by low dose of spironolactone and eplerenone refer to prevention of ischemia-reperfusion–induced activation of caspases 2, 3, and 9, therefore, implying targeted regulation of the intrinsic pathway. Such data confirmed that low-dose MR antagonists reduce infarct size and apoptosis in the reperfused myocardium due to prevention of apoptosis repressor with caspase recruitment domain degradation (Schmidt et al. 2009). Besides spironolactone and eplerenone, active metabolite of spironolactone canrenone in the form of potassium canrenoate was also shown to express cardioprotective properties in rat and rabbit animal models of myocardial ischemia reperfusion injury. Study protocol obtained 30 min of global ischemia and 2h of reperfusion period and also the onset of extended reperfusion of 72h. Interestingly, it has been proved that MR antagonists are cardioprotective as a postconditioning agents, when administered at the end of ischemia or 5 min prior to reperfusion. These effects are due to their impact on important signaling pathways involved in I/R injury pathogenesis, such as PKC (protein kinase C), adenosine receptors, PI3-kinase/Akt, and ERK.12. Suppression of this signaling pathways leads to suppression in mPTP opening at reperfusion period (Mahajan et al. 2018).

Beneficial effects of eplerenone 2-week pretreatment in I/R injury pathology were also demonstrated in diabetic conditions by Mahajan et al. This investigation highlighted the induction of the protective PI3K and Akt pathway and inhibition of the GSK-3β expressions as the crucial mechanism of eplerenone efficacy as a preconditioning agent. Their observations refer to preservation of myocardial structure, which is confirmed by the reduction of TUNEL-positive cells and inflammatory and necrotic histopathological changes. Eplerenone induced cardioprotection also included antiapoptotic action evaluated via reduction of Bax and increase in Bcl protein

expression. What is more, this cardiac protection was followed by prevention of oxidative stress, which has the major role in I/R injury pathology (Reddy et al. 2015; Van den Berg et al. 2016).

The role of chronic 8 week treatment with novel non-steroidal MR antagonist finerenone in postinfarction cardiac remodeling was investigated in recent years and compared to eplerenone as a standard, by using LAD model of myocardial infarction in rats. It was found that cardiodynamic parameters of the heart function such as dp/dtmax and dp/dt min were improved by finerenone in a dose of 1 mg/kg, but not by eplerenone. Additionaly finerenone treatment led to a decrease in the pro-inflammatory and pro-fibrotic marker osteopontin and plasma pro-BNP which indicates antihypertrophic activity of finerenone, with no significant influence on blood pressure when applied in this dosage (Kolkhof et al. 2014).

2. Clinical data

Despite large amount of evidence claiming MR antagonists beneficial properties in I/R injury in animal models, translation of these evidence into clinical trials remains a challenge. It is well known that the external validity of animal studies, in general, is limited due to biological differences between animals and humans. The main informations from clinical trials are presented in Table 2.

One of the first human studies which demonstrated results that are in sharp contrast with several previously described preclinical data confirming cardioprotective properties of MR antagonists on I/R injury was conducted in the 2016. by Van den Berg. This study included adult patients undergoing elective coronary artery bypass surgery, valve surgery or aortic surgery, with extracorporeal circulation. Atrial in vitro model was used in order to examine the effect of eplerenone on recovery of contractile heart function after I/R in 24 patients sample. This clinical trial showed diverse results and no significant limitation of I/R injury in human atrial tissue ex vivo by eplerenone acute administration (Van den Berg et al. 2016).

Another study was the first one to assess whether MR antagonists administered prior to reperfusion can provide beneficial effect against reperfusion injury and decrease infarct size in the clinical setting. Study involved a total of 70 adult patients with an acute STEMI, presenting in the first 12 hours after symptoms onset. The angiography inclusion criteria were TIMI 0 in a proximal left anterior descending, circumflex or right coronary

artery territory STEMI and K^+ serum level <5.0 mmol/l. Patients were treated with potassium canreanoat intravenously before restoration of blood flow. What is more, the patients were also treated with oral MR antagonists for 3 months in order to determine whether this approach could better post-MI LV remodeling in STEMI patients. No significant difference in the final infarct size was noticed at 3 months between the groups, also no difference in acute MI size (Bulluck et al. 2019).

Research group which investigated the impact of early additional eplerenone to recommended treatment on cardiovascular outcomes in STEMI over a 13 months follow-up has come to the conclusion that the addition of eplerenone during the acute phase of STEMI was safe and well-tolerated. This study included 1012 patients treated with placebo or eplerenone in a dose 25–50 mg once daily. One of the exclusion criteria was the glomerular filtration rate of equal or less to 30 mL per minute per 1.73 m^2 of body surface area because the eplerenone is contraindicated for use in these patients. The effect of eplerenone was particularly obvious in patients undergoing rapid reperfusion within 6h of symptom onset. The improvement in the primary outcome was mainly driven by a significant reduction of brain natriuretic peptide levels, which is a major prognostic factor for short-term and long-term prognosis in patients with MI (Montalescot G et al. 2014).

Hayashi et al. assessed the early use of spironolactone immediately post percutaneous transluminal coronary angioplasty in patients with anterior STEMI. Inclusion criteria were an electrocardiographic ST segment elevation 2 mV in 2 or more adjacent precordial leads and successful reperfusion therapy within 24 hours of the onset. The study included 150 patients and showed a reduction in postinfarct LV remodeling when spironolactone was used in combination with an angiotensin-converting enzyme inhibitor. Spironolactone significantly suppressed post-infarct elevation of PIIINP, a biochemical marker of myocardial fibrosis. The possible mechanism of the beneficial effects of spironolactone, aldosterone inhibitors, is reduced post-infarct collagen synthesis and progressive LV dilatation in patients with subacute MI (Hayashi et al. 2003).

Similar results were presented in a study by Kasama et al. Patients (97) with electrocardiographic ST-segment elevations >2 mV in two or more leads were treated with placebo or oral spironolactone (25mg) before primary coronary angioplasty. It was presented, for the first time, that the addition of spironolactone to standard treatment can improve cardiac sympathetic nerve activity and prevents LV remodeling in patients with a first

STEMI. Also, spironolactone can suppress cardiac collagen synthesis during the acute to subacute phase of STEMI after primary coronary angioplasty (Kasama et al. 2011).

The ALBATROSS trial investigated the effect of an early MR antagonists treatment, initiated within 72 hours of symptom, in AMI irrespective of the presence of HF or LV dysfunction. The patients were treated with potassium canrenoate in a dose of 200mg (IV bolus) followed by a daily 25-mg dose of spironolactone for 6 months but the benefit of early MR antagonists use in 1603 patients in that study was not shown (Beygui et al. 2016).

The beneficial effects of the addition of eplerenone to optimal medical therapy were also demonstrated in the study by Pitt et al. During a mean follow-up of 16 months, 3313 patients assigned to treatment 3 to 14 days after AMI were treated with eplerenone at the dose of 25 to 50 mg once daily. The study resulted in the improvement in survival and hospitalization rates among patients with AMI complicated by LV dysfunction and HF (Pitt et al. 2003).

The first clinical trial which compared nonsteroidal MR antagonists finerenone with eplerenone in patients with worsening heart failure and reduced ejection fraction and diabetes mellitus and/or chronic kidney disease was conducted on the 1066 patients. The study showed that the finerenone reduced levels of N-terminal pro-B-type natriuretic peptide to a similar extent to that of eplerenone with a good safety profile (Filippatos et al. 2016).

CONCLUSION

Taking into consideration all mentioned above, MR antagonists remained in the focus of research interest. The presence of MR in several cell types in cardiovascular system enables various mechanisms of cardioprotective effects of MR antagonists in myocardial ischemia reperfusion injury. Animal models confirmed the potency of MR antagonists as preconditioning and postconditioning agents in limiting infarct size and postinfarction remodeling. Since the data from clinical trials are still inconsistent, translation of these evidences into the practice remains a challenge. Therefore, further studies and the results of on-going clinical trials regarding MR antagonists administration in patients with acute myocardial infarction are being awaited with great interest.

CONFLICTS OF INTEREST

None of the authors of the present study has any actual or potential conflicts of interest to disclose, including financial, personal, or other relationships with specific persons or organizations.

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Tables

Table 1. Mechanistic insight of cardioprotection induced by MR antagonists in animal models

Preclinical studies	Treatment	Dose regimen	Effect on CVS	Mechanism	Sample
Schmidt K, et al. Cardioprotective effects of mineralocorticoid receptor antagonists at reperfusion(2009)	Eplerenone potassium Canrenoate	10 µM 50 µM	potassium canrenoate dose dependent ↓ infarct size	↓MPTP opening	Rabbit heart Open chest mice (cd73−/− or A2bAR−/− mice)
Mahajan UB Eplerenone pretreatment protects the myocardium against ischaemia/reperfusion injury through the phosphatidylinositol 3-kinase/Akt-dependent pathway in diabetic rats 2018	eplerenone	150mg/kg/day per os 14 days	↓I/R injury-induced oxidative stress ↓inflammation ↓apoptosis	↓MDA ↑SOD, CAT, GSH	STZ induced diabetic rats
Marcos F . et al. Mechanisms Involved in the Beneficial Effects of Spironolactone after Myocardial Infarction. 2013	Spironolactone	20 mg/kg/day 3 months	Alleviation of cardiac remodelling	↓colagen type I and III ↓periostin ↑ TIMP-1	Wistar rats
Mihailidou, et al. Glucocorticoids Activate Cardiac Mineralocorticoid Receptors During Experimental Myocardial Infarction. 2009	Spironolactone	10 nM and 1 mol/L Before ischemia, 10 min post stabilization	↓ infarct zone	↓apoptosis	Male Sprague-Dawley rats
Loan Le, et al. Low-Dose Spironolactone Prevents Apoptosis Repressor With Caspase Recruitment Domain Degradation	Spironolactone Eplerenone	10nmol/l and 100nmol/l	Prevention of apoptosis ↓ infarct zone	↓caspase 2, 3 and 9	Male Sprague-Dawley rats

During Myocardial Infarction. 2012					
Kolkhof P et al. Finerenone, a Novel Selective Nonsteroidal Mineralocorticoid Receptor Antagonist Protects From Rat Cardiorenal Injury. 2014	Finerenone Eplerenone	0.1, 0,3, 1 mg/kg/d 100 mg/kg/d	finerenone (1mg/kg) improved systolic and diastolic left ventricular function reduced cardiac hypertrophy by finerenone	Finerenone ↓heart weight-to-body weight ratio ↓proBNP	Wistar rats

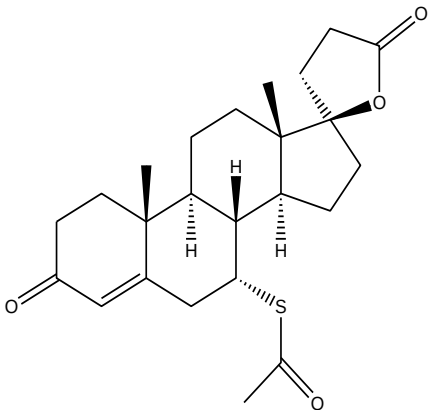
Table 2. Mechanistic insight of cardioprotection induced by MR antagonists in clinical trials

Clinical studies	Treatment	Dose regimen	Effect on CVS	Mechanism	Sample	Phase
Van den Berg, et al. Eplerenone does not limit ischemia–reperfusion injury in human myocardial tissue. 2016	Eplerenone	10 μM 10 min before simulated ischemia and continued throughout the experiment	No significant recovery of contractile function after I/R injury	/	Humans	II
MINIMIZE-STEMI trial 2019	Pottasium canrenoate Spironolactone	i.v. 200mg prior to reperfusion, followed by 20mg spironolactone per os	No reduction of infarct size	/	STEMI patients	II
Montalescot G, et al. Early eplerenone treatment in patients with acute ST-elevation myocardial infarction without heart failure: The Randomized Double-Blind Reminder Study. 2014	Eplerenone	25-50mg	↓BNP/NT-proBNP	/	STEMI patients	III
Hayashi M, et al. Immediate Administration of Mineralocorticoid Receptor Antagonist Spironolactone Prevents Post-Infarct Left Ventricular Remodeling Associated With Suppression of a Marker of Myocardial Collagen Synthesis in Patients With First Anterior Acute	Pottasium canrenoate Spironolactone	i.v. 200 mg prior to reperfusion, followed by 25mg spironolactone per os	↓postinfarct LV remodeling ↓post-infarct elevation of PIIINP	↓post-infarct collagen synthesis LV dilatation	anterior STEMI patients	II

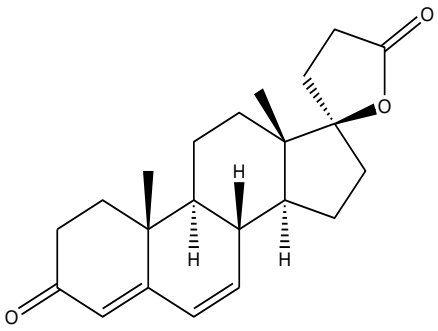
Myocardial Infarction						
Kasama S, et al. Effects of spironolactone on cardiac sympathetic nerve activity and left ventricular remodelling after reperfusion therapy in patients with first ST-segment elevation myocardial infarction.	Spironolactone	25mg	↓postinfarct LV remodeling Improve CSNA	↓post-infarct collagen synthesis	STEMI patients	II
The ALBATROSS Randomized Clinical Trial 2016	Pottasium canrenoate Spironolactone	i.v. 200 mg followed by 25mg spironolactone per os	No significant changes	/	Humans	III
Pitt B, et al. Eplerenone, a Selective Aldosterone Blocker, in Patients with Left Ventricular Dysfunction after Myocardial Infarction 2003	Eplerenone	25-50mg	↓morbidity ↓mortality	/	AMI	III
Filippatos G, et al. A randomized controlled study of finerenone vs. eplerenone in patients with worsening chronic heart failure and diabetes mellitus and/or chronic kidney disease 2016	Eplerenone Finerenone	25-50mg 2.5-20mg	↓NT-proBNP levels	/	Humans	II

Figures

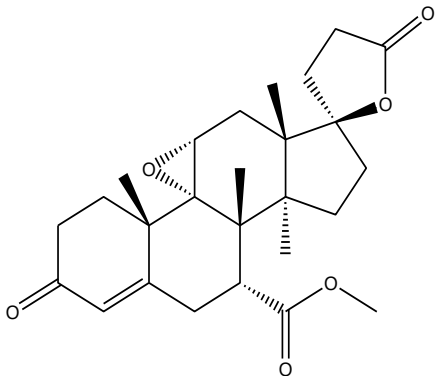
Figure 1. Cardioprotective effects of MR antagonists in the heart tissues post I/R injury



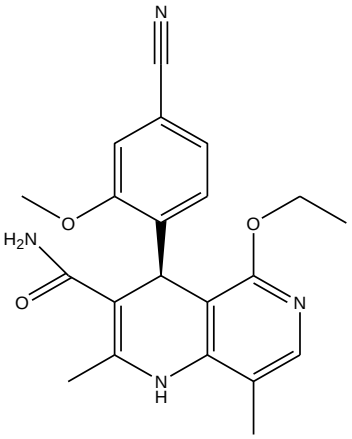
Spironolactone



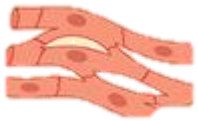
Canrenone



Eplerenone



Finerenone



fibroblasts

cardiomyocytes

endothelium

↓MMP
↓collagen

↓fibrosis

↓apoptosis
↓cell death
↓scar

↓K+ level

↓NADPH oxidase
↓superoxide

↓ROS
↑NO

↓LV remodelling

↓Arrhytmia

↓Oxidative stress

Attenuation of myocardial I/R injury