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Spironolactone and eplerenone are cardioprotective during early phase of ischemia in rats submitted to acute coronary occlusion

Espironolactona e eplerenona são cardioprotetoras durante a fase precoce de isquemia em ratos submetidos à oclusão coronária

Espirololactona y eplerenona son cardioprotectoras durante la fase temprana de isquemia em ratas sometidas a oclusión coronária aguda

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Abstract

Introduction: Mineralocorticoid receptor antagonists (MRAs) are effective in reducing left ventricle remodeling and sudden death after acute myocardial infarction (AMI). Objectives: MRAs *in vitro* display cardioprotective effects, independent of MR; however, it is unknown whether the rapid effects of MRAs are cardioprotective *in vivo*. This study evaluated the acute effects of spironolactone and eplerenone in the first minutes of AMI. Methods: Wistar Rats, submitted or not to bilateral adrenalectomy, were treated orally with spironolactone (20 mg/kg) or eplerenone (10 mg/kg), and submitted to the left coronary ligation, under anesthesia. Electrocardiogram (ECG) recordings were obtained to evaluate ST-T segment, QT, and QTc intervals. Arterial pressure was also measured before (baseline) and after coronary ligation. Results: Spironolactone or eplerenone given, one hour before coronary ligation, prevented ST-T segment elevation in adrenalectomized and non-adrenalectomized. QT interval analysis showed that MRAs

prevented its prolongation after coronary ligation. QT and QTc intervals remained similar to baseline and were smaller than the values displayed by the non-treated group. Animals treated with spironolactone, regardless of adrenalectomy, showed a 3-fold reduced mortality rates compared to the control group. Conclusion: MRAs display acute cardioprotective effects in early phase of AMI, which are independent of aldosterone.
Keywords: *Acute myocardial infarction; Spironolactone; Eplerenone; ECG; Cardioprotective*.

Resumo
Introdução: Os antagonistas do receptor de mineralocorticóide (MRAs) são eficazes na redução da remodelação do ventrículo esquerdo e morte súbita após infarto agudo do miocárdio (IAM). Objetivo: MRAs in vitro apresentam efeitos cardioprotetores, independente do MR; no entanto, não se sabe se os efeitos rápidos dos MRAs são cardioprotetores in vivo. Este estudo avaliou os efeitos agudos da espironolactona e da eplerenona nos primeiros minutos do IAM. Métodos: Ratos Wistar, submetidos ou não à adrenalectomia bilateral, foram tratados por via oral com espironolactona (20 mg / kg) ou eplerenona (10 mg / kg), e submetidos à ligadura da coronária esquerda, sob anestesia. Registros de eletrocardiograma (ECG) foram obtidos para avaliar o segmento ST-T, o intervalo QT e os intervalos QTc. A pressão arterial também foi medida antes (linha de base) e após a ligadura coronária. Resultados: Espironolactona ou eplerenona administrada uma hora antes da ligadura coronária preveniu a elevação do segmento ST-T em adrenalectomizados e não adrenalectomizados. A análise do intervalo QT mostrou que os MRAs impediram seu prolongamento após a ligadura coronária. Os intervalos QT e QTc permaneceram semelhantes à linha de base e foram menores do que os valores exibidos pelo grupo não tratado. Os animais tratados com espironolactona, independentemente da adrenalectomia, apresentaram taxas de mortalidade 3 vezes menores em comparação com o grupo controle. Conclusão: Os MRAs apresentam efeitos cardioprotetores agudos na fase inicial do IAM, que são independentes da aldosterona.
Palavras-chave: *Infarto agudo do miocárdio; Espironolactona; Eplerenona; ECG; Cardioprotetor*.

Resumen
Introducción: Los antagonistas de los receptores de mineralocorticoides (MRA) son efectivos para reducir el remodelado del ventrículo izquierdo y la muerte súbita tras un infarto agudo de miocardio (IAM). Objetivo: Los MRA in vitro tienen efectos cardioprotectores, independientes de la MR; sin embargo, no se sabe si los efectos rápidos de los MRA son cardioprotectores in vivo. Este estudio evaluó los efectos agudos de la espironolactona y la eplerenona en los primeros minutos del IAM. Métodos: Ratas Wistar, sometidas o no a adrenalectomía bilateral, fueron tratadas por vía oral con espironolactona (20 mg/kg) o eplerenona (10 mg/kg), y sometidas a ligadura de la arteria coronaria izquierda, bajo anestesia. Se obtuvieron registros de electrocardiograma (ECG) para evaluar el segmento ST-T, el intervalo QT y los intervalos QTc. También se midió la presión arterial antes (línea de base) y después de la ligadura coronaria. Resultados: La espironolactona o la eplerenona administradas una hora antes de la ligadura coronaria previnieron la elevación del segmento ST-T en pacientes adrenalectomizados y no adrenalectomizados. El análisis del intervalo QT mostró que los MRA evitaban su prolongación después de la ligadura coronaria. Los intervalos QT y QTc se mantuvieron similares a los basales y fueron inferiores a los valores exhibidos por el grupo no tratado. Los animales tratados con espironolactona, independientemente de la adrenalectomía, tuvieron tasas de mortalidad 3 veces más bajas en comparación con el grupo de control. Conclusión: Los MRA tienen efectos cardioprotectores agudos en la fase inicial del IAM, que son independientes de la aldosterona.
Palabras clave: *Infarto agudo de miocárdio; Espironolactona; Eplerenona; ECG; Cardioprotector*.

1. Introduction

Acute myocardial infarction (AMI) is among the leading causes of death and morbidity worldwide (Moran *et al*., 2014). The mineralocorticoid receptor antagonists (MRA), spironolactone and eplerenone, are effective to treat patients with mild or chronic heart failure (HF) and left ventricular (LV) dysfunction after AMI, mainly by reducing morbidity and mortality (Pitt *et al*., 2003; Zannad *et al*., 2010). Higher levels of aldosterone after AMI are associated with poor clinical outcomes, including mortality, sudden cardiac death, and heart failure (Beygui *et al*., 2009; Beygui *et al*., 2013). Aldosterone is associated with endothelial dysfunction, inflammation, ventricular hypertrophy, fibrosis, and cardiac remodeling (Weber 2001). Still, beneficial effects of early MRAs administration during AMI are supported by both experimental (Fraccarollo *et al*., 2008; Fraccarollo *et al*., 2015) and clinical studies (Montalescot *et al*., 2014).

The classical MRAs mechanism of action is the blockade of aldosterone action in the heart via mineralocorticoid receptors (MR) (Struthers 2004). The most comprehend mechanism of action of aldosterone in the heart is the genomic (slow) pathway, which is mediated by cytosolic intracellular MR receptors. This process involves translocation of aldosterone-MR complex to nucleus that acts as a transcriptional regulator that ultimately leads to protein synthesis (Rogerson *et al*., 2004).

Moreover, some protective effects attributed to MRAs occur even in the absence of abnormal levels of aldosterone. In the Randomized Aldactone Evaluation Study (RALES) and Eplerenone in post-myocardial infarct heart failure study (EPHESUS) aldosterone levels were physiological and MRA treatment displayed unquestioned benefits in HF patients (Pitt *et al*., 2003; Zannad *et al*., 2010). Additionally, low-dose spironolactone and eplerenone reduced the infarcted area and apoptosis in isolated heart of adrenalectomized or non-adrenalectomized rats (Mihailidou *et al*., 2009; Loan *et al*., 2012). However, it is not yet known whether the acute use of spironolactone and eplerenone are cardioprotective.

In this study, we evaluated whether MRAs, spironolactone or eplerenone, are cardioprotective during early phase of ischemia in a model of acute cardiac ischemia induced by coronary ligation in rats. Thus, animals were submitted to bilateral adrenalectomy to evaluate the effect of MRAs in absence of circulating endogenous aldosterone. It was then analyzed *in vivo* electrocardiogram (ECG) parameters, such as ST-T segment elevation, QT, and QTc intervals, as well as blood pressure and cardiac histological alterations of adrenalectomized and non-adrenalectomized rats subjected to cardiac ischemia.

2. Methodology

All the experimental procedures were approved by the Ethics Committee for Animal Use at the Federal University of Ouro Preto. (Protocol Nº. 2011/88). All procedures performed in studies involving animals were in accordance with the ethical standards of Brazilian animal welfare laws and guidelines.

Animals

Male Wistar rats (200 to 250 g) were obtained from the Vivarium of the Federal University of Ouro Preto (UFOP, Brazil). The animals were kept into polypropylene cages with free access to food and water with light and dark periods of 12/12 hours.

Bilateral adrenalectomy and dosage of serum aldosterone

The rats were anesthetized with ketamine (75 mg/kg) and xylazine (10 mg/kg) intraperitoneally (i.p). After shaving the lumbar region, a longitudinal skin and muscle incision was performed, the adrenal glands were removed, and the incision sutured. After that, the animals received flunixin (Banamine® - Intervet/Matercorp, Rio de Janeiro, RJ), an anti-inflammatory and analgesic drug, at 1 mg/kg/day. The animals were kept in cages with free access to food and water containing 0.9% NaCl [Drazen et al., 2004]. Only after a recovery period of 48 h, the animals underwent to left coronary ligation procedure. At the end of the experiment, blood samples were collected, and serum aldosterone was measured by radioimmunoassay (RIA aldosterone Assay, Cisbio Assays, Codolet, France).

Experimental Protocol

The rats were treated orally (per gavage) with spironolactone (20 mg/kg) or eplerenone (10 mg/kg). All the drugs were purchased from Sigma-Aldrich, USA. The doses used for all drugs were based on previous studies (Kobayashi *et al*., 2006; Silvestre *et a*l., 1999). The treatment was given to awaken animals 30 minutes before anesthesia for coronary ligation surgery. The animals were tracheostomized and connected to a small rodent respirator (SAR-840, USA) to allow artificial ventilation with room air (volume 1.5-2.5 ml/cycle and frequency of 45-50 cycles/min) (Bobryshev *et al*., 2009). The femoral artery and vein were catheterized to obtain the signal from the arterial pressure (AP) and to administer pancuronium bromide (1 mg/kg), a muscle relaxant, to assist with mechanical ventilation.

After these procedures, hypodermic needles were positioned subcutaneously to obtain ECG signal lead II and the femoral artery catheter was connected to a pressure transducer (TruWave; Edwards Life Sciences, USA). The method used to

induce myocardial ischemia was the left descending coronary ligation (Selye *et al*., 1960). The Sham group was submitted to the same surgical procedure without coronary ligation. The control group was submitted to coronary ligation and treated with vehicle. The entire procedure from the drug treatment until coronary ligation took approximately 60 to 70 minutes. Then, the ECG signal was continuously obtained in anesthetized rats before (baseline) and during the 60 minutes after left coronary ligation. After one hour of ligation procedure, the animals were euthanized, and the heart was removed for histological analysis.

Cardiovascular parameters determination

To obtain the arterial pressure (AP) and electrocardiography (ECG) signal parameters, a real-time system of signal conditioner was used at a frequency of 1200 Hz, using an analog-digital converter board of 12-bit resolution (Daqboard 2000, USA). To analyze the systolic blood pressure (SBP), diastolic blood pressure (DBP), and ECG parameters used 2 seconds long segments, that correspond to 8 to 12 heart beats, before (baseline), and 60 minutes after coronary ligation was extracted. ECG signal, baseline, 10, 30, and 50 minutes after coronary ligation, was used to measure RR interval to obtain the heart rate (HR), QT interval and QTc interval, that is the QT corrected by HR according to Fridericia's formulae: $QTc = QT/(RR)^{0.33}$. The QT interval is widely used in experimental and clinical studies to identify cardiac risk, as susceptibility to arrhythmias and cardiac ischemia (Baillard *et al*., 2000). The ST segment elevation, a parameter that indicates the myocardium ischemia, was also evaluated. This parameter was calculated by the area under the curve of ST-T segment. From lead II ECG signal a horizontal line was drawn between the beginning of the inclination of S wave and the end of T wave when the latter reaches the isoelectric point (Resende *et al*., 2012). To determine the area, the software Analyzer 7.0/Hemolab Data Acquisition software 14.2 was used.

Histological Analysis

The hearts were fixed into a 10 % formalin solution. The ventricles were separated and sectioned into three transverse sections; dehydrated with alcohol in increasing concentrations; diaphonized with xylene and embedded in paraffin. Paraffin sections of approximately four micrometers thick were obtained in a microtome, fixed on glass slides, and then stained with hematoxylin and eosin (Acikel *et al*., 2005).

Statistical Analysis

For the analysis of ECG parameters and AP the comparison between the groups at different times was performed using one-way ANOVA followed by Bonferroni post-test. When the analysis was conducted within different groups at the same time point, two-way ANOVA followed by Tukey posttest was used. Fisher test was used to identify mortality differences. Statistical analysis was performed with GraphPad Prism Project (version 6.0) software. Significant differences at p value < 0.05 were considered.

3. Results

Serum aldosterone after adrenalectomy

Serum aldosterone levels were dramatically reduced after the adrenal glands removed compared to the Sham group (93 ± 25.7 ng/dl). No difference was observed between the groups submitted solely to coronary ligation (2 ± 0.6 ng/dl) compared to groups submitted to the same procedure and treated with either spironolactone (1 ± 0.7 ng/dl) or eplerenone (1 ± 0.5 ng/dl).

Blood pressure and ECG parameters

Table 1 presents SBP and DBP data of all experimental groups, both baseline and after 60 minutes of coronary ligation. In non-adrenalectomized rats, we observed a significant reduction of SBP and DBP in the vehicle-treated group after coronary ligation. No significant difference between SBP and DBP was observed in spironolactone- or eplerenone-treated groups submitted to coronary ligation compared to baseline of the same group or vehicle treatment; thus, showing the ability of both drugs to prevent arterial pressure reduction observed after ischemia. In adrenalectomized rats, SBP and DBP were significantly lower in all groups submitted to coronary ligation despite different treatments compared to baseline and compared to non-adrenalectomized with the same treatment (Table 1).

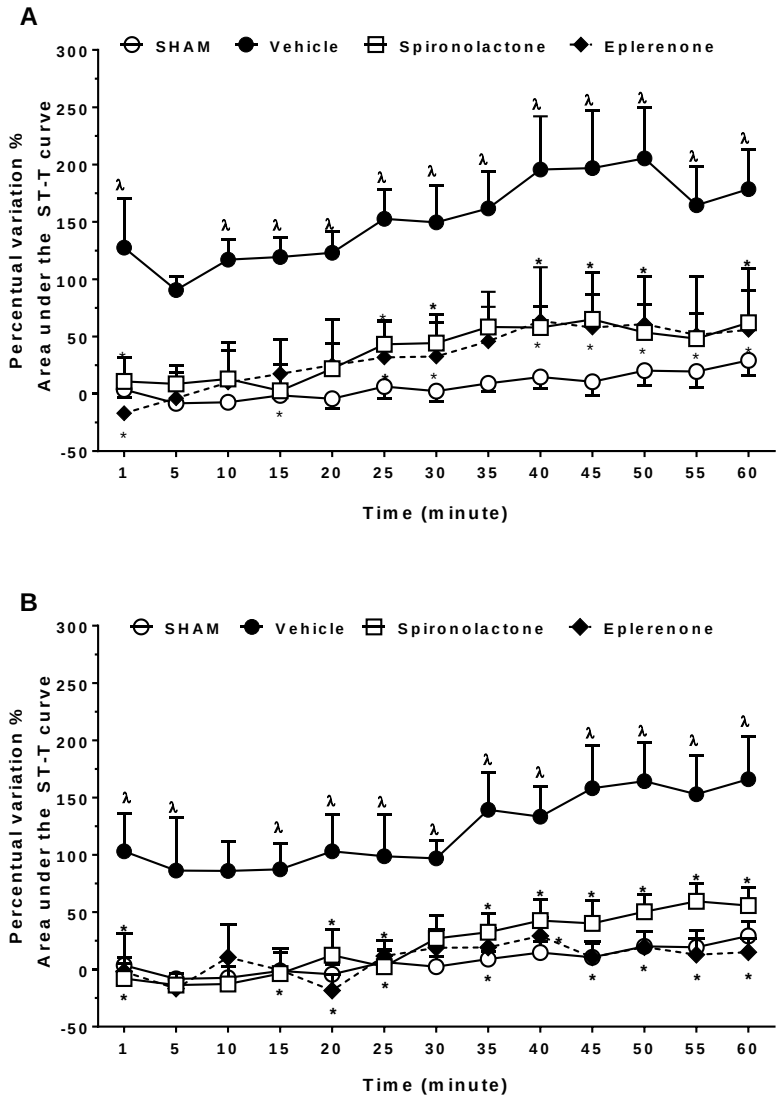
Table 1: Absolute values of SBP and DBP of all experimental groups before (baseline) and after coronary ligature.

Treatment		Non-Adrenalectomy		Adrenalectomy	
		Baseline	**After**	**Baseline**	**After**
Sham	SBP	120 ± 8.1	122 ± 1.6	-	-
	DBP	88 ± 6.3	89 ± 1.3	-	-
Vehicle	SBP	108 ± 8.2	83 ± 8.5*	94 ± 8.1	50 ± 4.2*$^{\#}$
	DBP	79 ± 5.9	55 ± 3.9*	70 ± 6.1	39 ± 4.1*$^{\#}$
Spironolactone	SBP	107 ± 5.1	87 ± 12.9	88 ± 8.9	43 ± 6.5*$^{\#}$
	DBP	77 ± 4.9	55 ± 3.9	68 ± 7.3	31 ± 4.4*$^{\#}$
Eplerenone	SBP	105 ± 7.3	93 ± 7.7	84 ± 3.6	46 ± 5.0*$^{\#}$
	DBP	72 ± 5.6	63 ± 12.7	63 ± 3.2	33 ± 3.8*$^{\#}$

ANOVA Two-way followed by Bonferroni posttest: $^{\#}P<0.05$ compared to baseline of non-adrenalectomy group. Paired *t* student test: *P<0.05 compared to baseline of the same group. Source: Authors.

ECG signal lead II was used for the quantitative analysis of ST segment elevation. Figure 1 shows the percentage variation of area under the curve ST-T during one hour after coronary ligation procedure. Coronary ligation of non-adrenalectomized rats without treatment promoted ST-T segment elevation compared to the Sham group (Figure 1A). The MRAs, spironolactone or eplerenone orally administered one hour before ligation, prevented ST segment elevation (Figure 1A) compared with the vehicle treated the coronary ligation group and the response was also similar to the Sham group. The response profile observed in adrenalectomized animals subjected to coronary ligation without treatment and treated with spironolactone or eplerenone was also similar (Figure 1B), showing clearly that MRAs drugs could prevent ischemia alterations of ECG signal even in the absence of aldosterone.

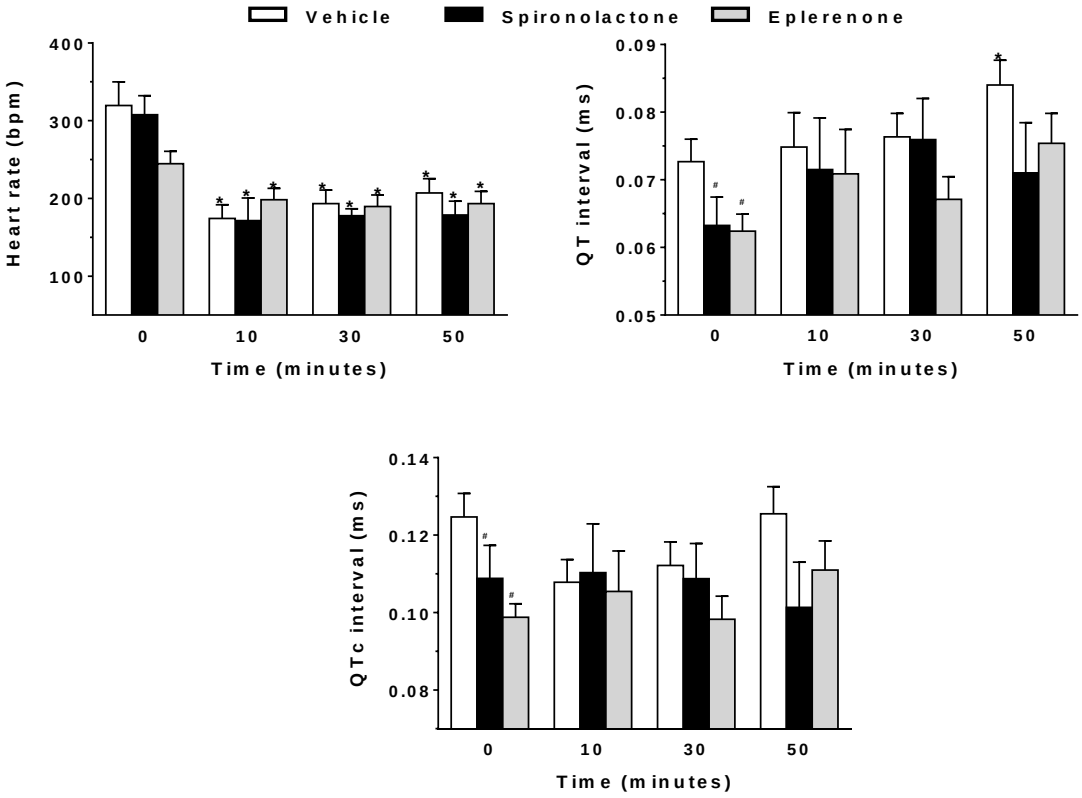
Figure 1. Percentual variation of the area under the ST-T curve from ECG of rats treated with spironolactone or Eplerenone subjected to coronary ligation.



A) Non-adrenalectomized animals; B) Adrenalectomized animals. Values are expressed as mean ± SEM two-way ANOVA followed by Bonferroni Posttest. *p < 0.05 compared to coronary ligation group vehicle treated; λ p < 0.05compared to the Sham group. N = 3 – 6. Source: Authors.

Regarding HR, significant bradycardia was observed one hour after coronary occlusion in all groups compared to baseline values. However, it was not observed difference in HR between any groups. Considering that prolonged QT interval of ECG as an arrhythmia and sudden cardiac death predictor (Schwartz & Wolf 1978), this parameter was analyzed, being corrected by HR. QT and QTc intervals of ECG analysis were performed at baseline 10, 30, and 50 minutes after coronary ligation (Figure 2). Baseline values of QT and QTc intervals were reduced when rats were treated with spironolactone (60 ± 3.1 ms and 101 ± 5.1 ms, respectively, for QT and QTc) or eplerenone (62 ± 2.5 ms and 99 ± 3.5 ms, respectively, for QT and QTc) compared to vehicle treatment (73 ± 3.3 ms and 125 ± 6.1 ms, respectively, for QT and QTc). Coronary ligation of vehicle-treated rats induced QT interval prolongation observed 50 minutes after and this effect was not observed for any treatment or for QTc interval, thus showing the ability of MRA treatment to prevent this alteration. Although we observed a decreasing trend, the lack of QTc alteration was probably due to intense bradycardia induced by the surgical procedure.

Figure 2. Heart rate, QT, and QTc intervals of rats treated with spironolactone or eplerenone subjected to coronary ligation.



Values are expressed as mean ± SEM. Two-way ANOVA followed by Bonferroni post-test. *p < 0.05 compared to baseline (zero). #p < 0.05 differences compared to vehicle treatment. N = 4 – 6. Source: Authors.

The mortality after coronary ligation was 30.6 % and 37.5 % of vehicle treated non-adrenalectomized and adrenalectomized animals, respectively, in less than one hour after (Table 2). Animals treated with eplerenone regardless of adrenalectomy showed similar mortality rate compared to the vehicle treated group (Table 2). Non-adrenalectomized and adrenalectomized rats treated with spironolactone showed approximately 3-fold reduction of mortality rate within one hour after ligation.

Table 2. Mortality rate of Wistar rats submitted to coronary ligature and different treatments.

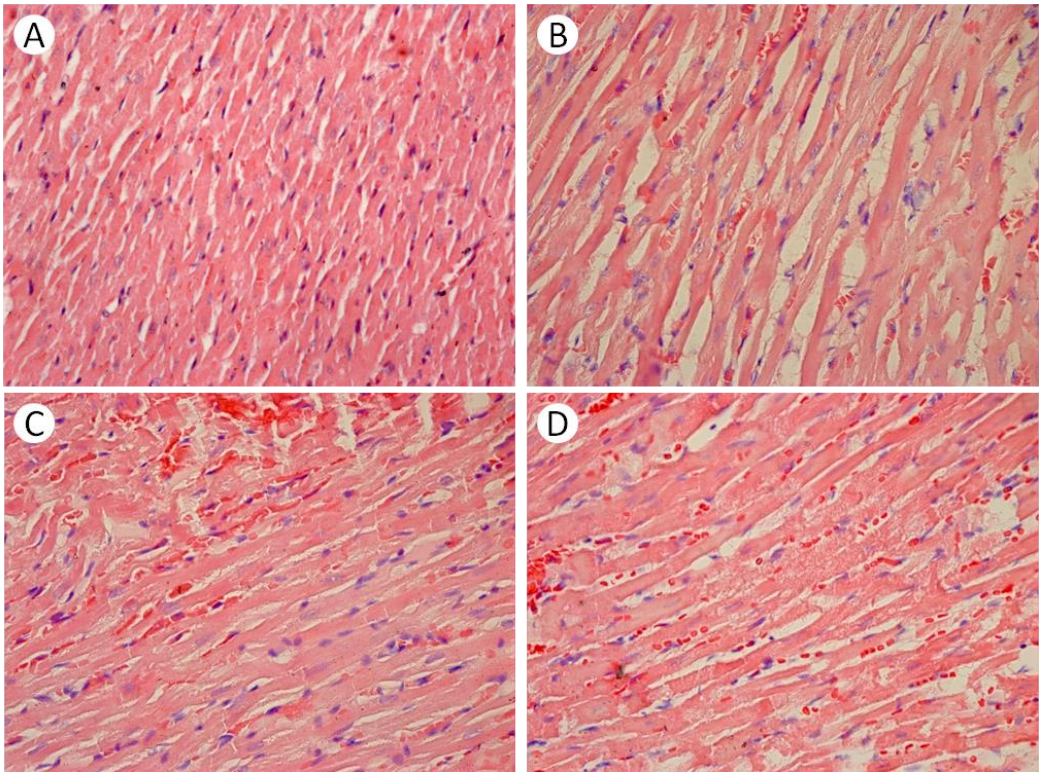
	Non-Adrenalectomy		Adrenalectomy	
Treatment	**Absolute values (n death/total)**	**Rate (%)**	**Absolute values (n death/total)**	**Rate (%)**
Vehicle	11/36	30.55	9/24	37.5
Spironolactone	1/12	8.3*	2/14	14.3*
Eplerenone	3/9	33.33	4/10	40.0

Groups were submitted to coronary ligature and mortality evaluated until one hour after. *Fisher test indicates the statistical differences. Source: Authors.

Histological Analysis

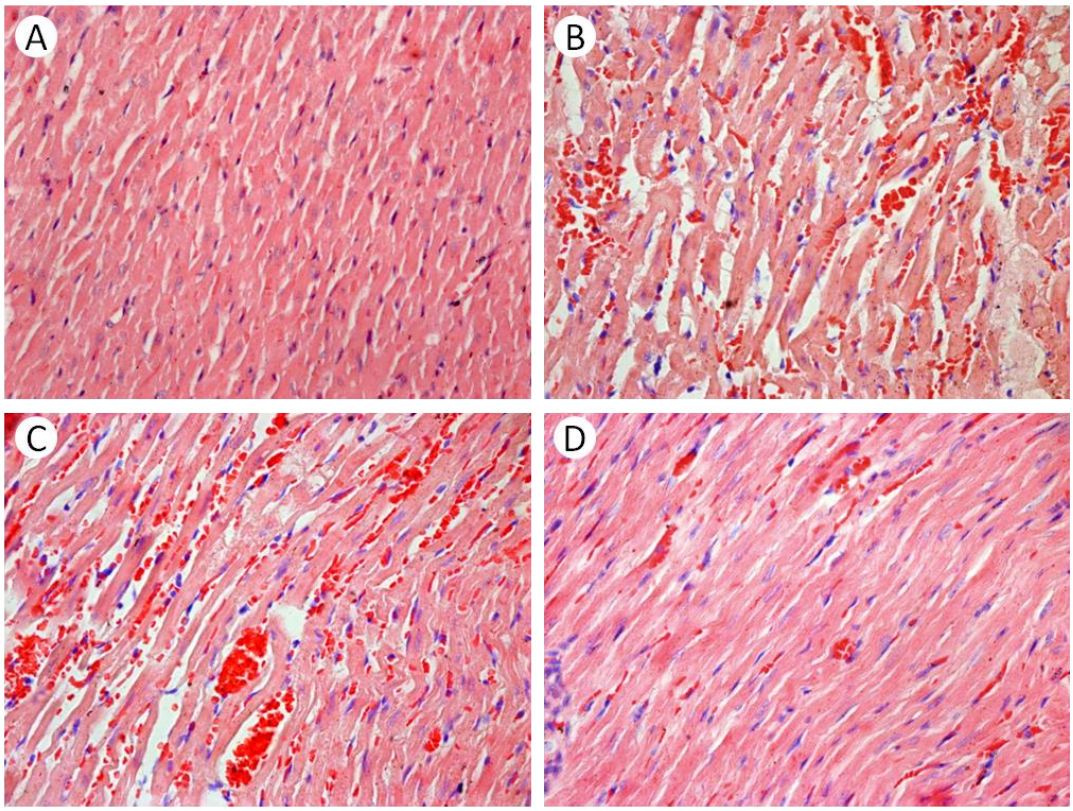
Histological sections were stained with hematoxylin and eosin, and we observed that the cardiac muscle fibers from the groups submitted to coronary ligation display abnormalities, presence of necrosis, interstitial edema, and absence of cross-cutting striations. These changes were observed in all groups irrespective of treatment and adrenalectomy. No edema or necrosis was observed in the Sham group (Figures 3 and 4).

Figure 3. Representative micrographs of hematoxylin and eosin staining showing histological changes of the rat heart after coronary ligation of non-adrenalectomized groups.



(A) SHAM; (B) Vehicle; (C) Spironolactone 20 mg/kg; (D) Eplerenone 10 mg/kg (40x). Source: Authors.

Figure 4: Representative micrographs of hematoxylin and eosin staining showing histological Changes of the rat heart after coronary ligation of adrenalectomized groups.



(A) SHAM; (B) Vehicle; (C) Spironolactone 20 mg/kg; (D) Eplerenone 10 mg/kg (40x). Source: Authors.

4. Discussion

This study demonstrated the effects of MRAs, spironolactone and eplerenone, at first hour after myocardial ischemia in rats. These two drugs could prevent ST-T increase and QT interval prolongation induced by coronary ligation in the presence or absence of circulating endogenous aldosterone.

The elevation of the ST-T segment is an ECG marker of cardiac ischemia, and the reduction of this parameter indicates cardioprotection (Coppola *et al*., 2013; Birnbaum & Drew 2003). Our data showed that treatment with spironolactone or eplerenone prevents ST-T segment elevation in non-adrenalectomized and adrenalectomized rats submitted to ischemia, suggesting a possible reduction of infarct area. No significant changes were found in hematoxylin and eosin histological analysis, but these data are not conclusive. Our AMI model has permanent left anterior descending artery ligation for 1 h. The time required for completely necrosis of cardiac cells is about 30-90 minutes in rats (Jugdutt 1993). HE staining analysis is not the most precise method to quantify ischemia injuries in AMI. The infarct size and the area at risk should be performed in the future. Previously, studies demonstrated that spironolactone or eplerenone infusion before and immediately after ischemia could reduce the infarcted area in isolated rat heart (Mihailidou *et al*., 2009; Loan *et al*., 2012; Chai *et al*., 2005). This protective action can be explained since MRAs prevent cardiac apoptosis by inhibiting the degradation of apoptosis repressor with caspase-recruiting domain, a multifunctional inhibitor of apoptosis (Loan *et al*., 2012). These findings provide direct evidence that MRAs have effects independent of MR blockade.

Another electrocardiography alteration associated with AMI is a prolongation of QT interval duration. The prolonged QT interval leads to electrical conduction disorder that alters the ventricular repolarization and increases the likelihood of ventricular tachyarrhythmia and sudden death (Passman & Kadish 2001). MRAs are well-known drugs that reduce the rate of

sudden cardiac death in heart failure patients (Pitt *et al*., 2003). Eplerenone treatment also showed significant reductions in sudden death and total mortality in the early post-MI period (Pitt *et al*., 2005). Spironolactone improved QT interval dispersion (Yee *et al.*, 2001) and, along with the angiotensin-converting-enzyme inhibitor, reduced the arrhythmic score post-myocardial infarction (Beck *et al*., 2001). In fact, aldosterone has pro-arrhythmic effects. MR activation leads to arrhythmia by increasing tissue remodeling and fibrosis as well as alteration in the electrophysiology of the cardiomyocytes, and leads to early and delayed after-depolarization, which is a major cause of life-threatening arrhythmia (Gravez *et al*., 2013). Therefore, these data may explain, sudden death decrease of MRA treated patients, but do not explain why MRAs display cardioprotective effects in patients with normal levels of aldosterone. In fact, our study demonstrated antiarrhythmic effects of MRA in the first phase of AMI that prevents QT interval prolongation fifty minutes after coronary ligation, which suggests that MRAs have acute arrhythmogenic effects.

Additionally, the group treated with spironolactone – regardless of adrenalectomy – displayed a 3-fold reduction in the mortality rates compared to the non-treated and eplerenone-treated groups, probably due to an antiarrhythmic effect. Previous studies have shown that animals subjected to coronary ligation and treated with spironolactone during 7 days show reduced mortality compared to the control group (Song *et al*., 2011). This cardioprotective effect was attributed to the inhibition of ischemia-induced ventricular premature beats by reducing the expression hyperpolarization-activated cyclic nucleotide–gated (HCN) isoform 4 (Song *et al*., 2011). It is unclear why eplerenone did not reduce the mortality rate. This result is contrary to the one found in mice submitted to coronary ligation as survival rate was significantly increased in the eplerenone group compared with the spironolactone group (Fraccarollo *et al*., 2008).

The acute cardioprotective mechanisms of spironolactone and eplerenone are yet unknown. Previously, we have demonstrated *in vitro* that adding spironolactone or eplerenone for 30 min increases the intracellular levels Ca^{2+}, cAMP (only for spironolactone), cGMP, and increase cardiomyocyte proliferation, as well as reduce fibroblast proliferation (Hermidorff *et al*., 2015). The activation of these mediators can be associated with unknown membrane-receptor (Hermidorff *et al*., 2015; Hermidorff *et al*., 2017). Rapid effects of aldosterone have been associated with GPER-1 receptor and it been demonstrated that MRAs inhibited partially GPER-mediated aldosterone effects in the vascular system (Gros *et al*., 2011; Gros *et al*., 2013); however, in the heart, this antagonism was not observed (Ashton *et al*., 2015).

In addition, eplerenone or canrenoate treatment increased phosphorylation of AKT and ERK1/2 at reperfusion in the isolated rat hearts and the protection was inhibited by co-treatment with inhibitors of the adenosine receptor, protein kinase C, PI3-kinase, and ERK (Schmidt *et al*., 2010). Adenosine appears to have an important role in these pathways. Stimulation of membrane-bound adenosine receptors (A_1, A_{2A}, A_{2B}, and A_3) reduces isquemia-reperfusion injury (Paiva *et al.*, 2009) and prevents cardiac arrhythmias (Riksen & Rongen 2012). However, in a clinical study, eplerenone does not augment extracellular adenosine formation in healthy human subjects (van den Berg *et al*., 2014). Another line of thought to explain our findings is the fact that MR antagonists may act as inverse agonists, occupying this receptor, triggering opposite responses of what an agonist would do (Mihailidou *et al*., 2009). Thus, it is still early for any definitive conclusion, as more studies need to be performed regarding the direct pathways activated by MRAs in the cardiovascular system.

5. Conclusion

We demonstrated in this work that both eplerenone and spironolactone prevent ECG changes induced by ischemia in adrenalectomized rats. It has also been shown that these effects are independent of the antagonistic action of these drugs on the aldosterone receptor (MR). Our data, added to what exists in the literature, clearly demonstrate the beneficial interference of MRAs already in the initial phase of acute myocardial infarction. Seeking to understand more about the mechanisms of action

of these pharmacos, at the molecular level, is a necessary task. This understanding will allow the design of new pharmacos, more specific, more efficient and certainly with fewer unwanted effects.

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