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Original Article

Additive Effects of Esaxerenone, a Nonsteroidal Mineralocorticoid Receptor Blocker, on Cardioplegic Arrest in Rat Hearts

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Purpose: Esaxerenone, a mineralocorticoid receptor blocker, attenuates global ischemia-induced myocardial damage and coronary endothelial dysfunction. This study aimed to determine whether esaxerenone exerted cardioprotective effects against cardioplegic arrest in Wistar rat hearts.

Methods: Isolated male Wistar rat hearts aerobically perfused via the Langendorff method for 20 min were randomly allocated to the Control (n = 6; perfused for an additional 10 min and subjected to no treatment) or Esax (n = 6; perfused with 0.1 μmol/L esaxerenone in perfusate for 10 min before ischemia) groups. Hearts in both groups were perfused with St. Thomas' Hospital No. 2 solution (STH2) for 2 min and subjected to 28 min of global ischemia. The recovery of left ventricular developed pressure (LVDP) and total troponin T leakage were measured after reperfusion.

Results: The final recovery of LVDP (expressed as a percentage of pre-ischemic value) in the Control and Esax groups was 50.8 ± 3.5% and 62.1 ± 5.6%, respectively (p <0.05, Esax vs. Control). The total troponin T leakage in the Control and Esax groups was 138.8 ± 18.5 ng/g heart wt and 74.3 ± 18.6 ng/g heart wt, respectively (p <0.05, Esax vs. Control).

Conclusion: The administration of esaxerenone before cardioplegic arrest enhanced the cardioprotective effect exerted by STH2.

Keywords: esaxerenone, St Thomas' Hospital No. 2 solution, cardioplegic arrest, myocardial protection

Introduction

Myocardial deterioration, a major complication of cardiac surgery, is affected by multiple factors; however, ischemic insult and reperfusion injury have been identified as major contributing factors. Although several cardioplegic solutions have been used worldwide,[1)] hyperkalemic cardioplegic solutions, such as St. Thomas' Hospital No. 2 (STH2) solution, remain one of the gold standard methods for protecting the myocardium against ischemic insult. Elevated extracellular K^+ levels shift the resting cell membrane potential of myocytes and inactivate the fast Na^+ channels, thereby blocking the conduction of the myocardial action potential. This blockade results in a "depolarized" arrest. However, depolarized arrest can result in myocyte

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damage as an inward non-inactivating Na^+ window current is generated at less negative membrane potentials. This leads to intracellular Na^+ loading, followed by Ca^{2+} loading of the myocyte, resulting in contracture and cell death.[2] Thus, developing better intraoperative myocardial protection additives or alternatives to hyperkalemic cardioplegic solutions is imperative.

The activation of the aldosterone/mineralocorticoid receptor contributes to multiple harmful molecular mechanisms involved in the development of heart failure. Steroidal mineralocorticoid receptor blockers (MRBs), spironolactone, and eplerenone are effective in reducing cardiovascular mortality and morbidity in patients with chronic heart failure who have a reduced left ventricular ejection fraction.[3,4] Moreover, these drugs also induce a significant reduction in the incidence of postoperative atrial fibrillation in patients undergoing cardiac surgery.[5] Nevertheless, they remain underutilized owing to the risk of severe adverse events, such as hyperkalemia and worsening of kidney function.

Esaxerenone, a potent and selective non-steroidal MRB, has pharmacological properties distinct from those of steroidal MRBs.[6] When used for the treatment of hypertension, esaxerenone induces reverse remodeling in patients with heart failure who have a preserved ejection fraction.[7] Moreover, esaxerenone may be effective in reducing brain natriuretic peptide levels in patients with heart failure who have a reduced ejection fraction.[8]

Myocardial protection in response to simple global ischemia can be achieved via the pre-ischemic administration of esaxerenone.[9] Attenuation of coronary endothelial ischemia–reperfusion injury may be one of the mechanisms underlying the protective effect of esaxerenone.[9] This study aimed to investigate whether the pre-ischemic administration of esaxerenone could attenuate ischemic and reperfusion injury of the heart after cardioplegic arrest and enhance the myocardial protective effect of conventional hyperkalemic cardioplegia. A clinically feasible method to improve myocardial protection via cardioplegic arrest during cardiac or aortic surgery can be developed based on the findings of this study.

Materials and Methods

Ethics statements

All animals received humane care in accordance with the guidelines stated in the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" published by the National Institute of Health (NIH; NIH publication no.: 85-23, revised 1996). The Animal Ethics Committee of the Nippon Medical School approved this study (no.: 2020-079).

Animals

In all, 12 male Wistar rats (Oriental Yeast Co., Ltd., Tokyo, Japan) weighing 240–300g were anesthetized with pentobarbital (50 mg/kg, intraperitoneally) and anticoagulated with heparin (1000 IU/kg, intravenously). The exclusion criteria are described in the subsequent section.

Heart isolation and perfusion

The rat hearts were excised and immersed in the cold (4°C) Krebs–Henseleit bicarbonate buffer (KHB). The aorta was cannulated subsequently, and the heart was perfused with KHB via the Langendorff method under constant pressure (80 mmHg) at 37°C, as described in a previous study.[10] A saline-filled vinyl balloon was inserted into the left ventricle via the left atrium. The left ventricular pressure was measured using a pressure transducer connected to the balloon. The balloon volume was adjusted such that a left ventricular end-diastolic pressure (LVEDP) of 3–8 mm Hg was achieved. All hearts were subjected to 20 min of aerobic perfusion, and the following baseline readings were recorded: left ventricular systolic pressure (LVSP, mmHg), LVEDP (mmHg), heart rate (beats/min), and coronary flow (mL/min). The left ventricular developed pressure (LVDP) was calculated using the following formula:

$$LVDP = LVSP - LVEDP.$$

Heart with LVDP, heart rate, and coronary flow beyond the acceptable range (>100 mmHg, >200 beats/min, and 10–20 mL/min, respectively) at the time of baseline recordings were excluded.

Perfusion medium and drugs

KHB comprised 118.5 mmol/L of NaCl 25.0 mmol/L of $NaHCO_3$; 4.8 mmol/L of KCl, 1.2 mmol/L of $MgSO_4$, 1.18 mmol/L of KH_2PO_4, 1.4 mmol/L of $CaCl_2$, and 11.0 mmol/L of glucose. KHB was prepared daily and filtered through a 5-μm cellulose nitrate filter.

STH2 (Miotector; Mochida Pharmaceutical Co., Ltd., Tokyo, Japan), a conventional hyperkalemic cardioplegia agent, comprised 110.0 mmol/L of NaCl, 16.0 mmol/L of $MgCl_2 \cdot 2H_2O$, 16.0 mmol/L of KCl, 1.2 mmol/L of $CaCl_2 \cdot 2H_2O$, and 10.0 mmol/L of $NaHCO_3$. The solution

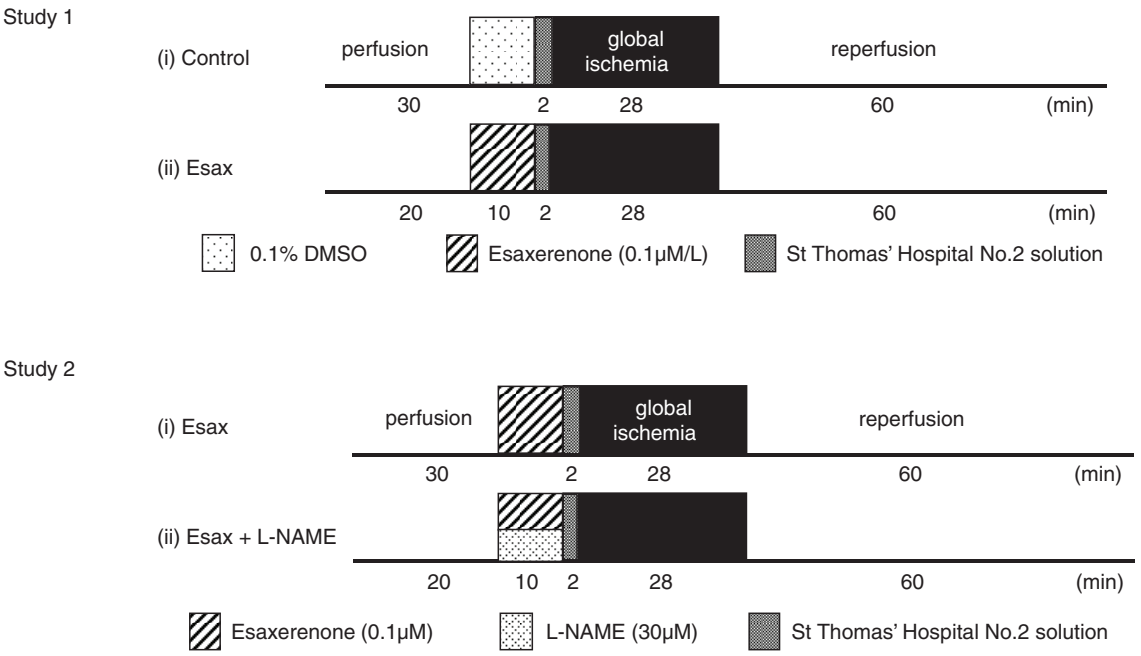

Fig. 1 Experimental perfusion protocol. The hearts were aerobically perfused with KHB buffer via the Langendorff method at a constant pressure of 80 mmHg before and after ischemia. (study 1) (i) Control: 60-min reperfusion with KHB (only STH2 treatment) (ii) Esax: infusion with 0.1 μmol/L esaxerenone-dissolved KHB for 10 min before ischemia. (study 2) (i) Esax: infusion with 0.1 μmol/L esaxerenone-dissolved KHB for 10 min before ischemia. (ii) L-NAME + Esax: infusion with 0.1 μmol/L esaxerenone-dissolved KHB and 30 μmol/L L-NAME for 10 min before ischemia. STH2: St. Thomas' Hospital No. 2 solution; L-NAME: N(x)-nitro-L-arginine monomethyl ester; KHB: Krebs-Henseleit bicarbonate.

was prepared daily, and the pH was adjusted to 7.8 at 37°C. The solution was filtered through a 5-μm cellulose nitrate filter before use.

A material transfer agreement for esaxerenone was signed by the Nippon Medical School and Daiichi Sankyo Co., Ltd., prior to commencing this study. Esaxerenone (0.1 μmol/L) was dissolved in dimethyl sulfoxide (DMSO; final concentration, less than 0.1%). The concentration was determined based on the findings of a previous study.[9] N(x)-nitro-L-arginine monomethyl ester (L-NAME), a nonspecific nitric oxide synthase (NOS) inhibitor, was purchased from R&D Systems, Inc (Minneapolis, MN, USA).

Perfusion protocol

Figure 1 illustrates the perfusion protocol. In study 1, hearts were equilibrated via a 20-min aerobic KHB perfusion at 37°C, regardless of the perfusion protocol used, and randomly allocated to the Control or Esax groups (n = 6 per group). The hearts allocated to the Control group were perfused for an additional 10 min with KHB, including DMSO only, and received no treatment throughout the protocol (only STH2 treatment). The hearts allocated to the Esax group were perfused with 0.1 μmol/L esaxerenone in perfusate for 10 min before ischemia. The hearts were subjected to a 2-min infusion of STH2 and 28 min of global ischemia at 37°C subsequently, which was induced by eliminating flow by clamping the tube, and re-perfused for 60 min after global ischemia. In study 2, hearts were equilibrated via a 20-min aerobic KHB perfusion at 37°C, regardless of the perfusion protocol used, and randomly allocated to the Esax or L-NAME + Esax groups (n = 5 per group). The hearts allocated to the Esax group were perfused with 0.1 μmol/L esaxerenone in perfusate for 10 min before ischemia. The hearts allocated to the L-NAME + Esax group were perfused with 0.1 μmol/L esaxerenone and 30 μmol/L L-NAME in perfusion for 10 min before ischemia.[10] The hearts were subjected to a 2-min infusion of STH2 and 28 min of global ischemia at 37°C, which was subsequently induced by eliminating flow by clamping the tube and re-perfused for 60 min after global ischemia.

Expression of results

The post-ischemic recovery of LVDP, heart rate, and coronary flow were expressed as percentages of the baseline values. LVEDP was expressed as an absolute value

Table 1 Baseline values and final percentage of recovery of heart rate, LVDP, coronary flow, and final LVEDP of the Langendorff-perfused rat hearts

	Control	Esax	p-value
Heart rate			
Baseline (beats/min)	302.5 ± 20.0	297.5 ± 44.4	0.807
Recovery (%)	89.1 ± 5.7	93.6 ± 15.0	0.513
LVDP			
Baseline (mmHg)	145.8 ± 10.4	132.3 ± 21.7	0.199
Recovery (%)	50.8 ± 3.5	62.1 ± 5.6	0.001
Coronary flow			
Baseline (mL/min)	17.6 ± 1.9	17.5 ± 3.2	0.966
Recovery (%)	55.5 ± 7.4	62.0 ± 11.3	0.266
LVEDP			
Baseline (mmHg)	4.7 ± 0.6	4.3 ± 1.3	0.512
Recovery (mmHg)	50.0 ± 5.4	34.2 ± 8.5	0.003

Control: additional 10-min perfusion with Krebs–Henseleit bicarbonate buffer (KHB; treatment only with St. Thomas' Hospital No. 2 solution); Esax: infusion with 0.1 μmol/L esaxerenone-dissolved KHB for 10 min before ischemia and treatment with St. Thomas' Hospital No. 2 solution

LVDP: left ventricular developed pressure; LVEDP: left ventricular end-diastolic pressure

(mmHg). Myocardial edema was evaluated by measuring the water content in each heart using microwaves, as described in a previous study.[11] Coronary effluents were collected during reperfusion, and the total troponin T levels expressed as ng/g heart weight (g wt) were measured using an electrochemiluminescence immunoassay (Roche Diagnostics K.K., Tokyo, Japan) to evaluate myocardial damage.

Statistical analyses

All data are presented as means ± standard deviation. Comparisons between the continuous variables of the two groups were made using one-way analysis of variance (ANOVA) or two-way repeated-measures ANOVA with correction by linear regression analysis, as appropriate. A *post hoc* analysis was performed using Tukey's test, which allowed for multiple comparisons if significant differences were observed.

All statistical tests were two-tailed, and a p-value of <0.05 was considered statistically significant. All statistical analyses were performed using JMP, version 10.0 (SAS Inc., Cary, NC, USA).

Results

Recovery of cardiac function

Table 1 presents the baseline and final recovery measurements for all parameters. The LVDP recovery of the Control group was approximately 50% of that in the pre-ischemic condition. The Esax group recovered rapidly and attained values higher than those of the Control group. However, the values plateaued within 20 min (**Fig. 2A**). The final recovery of LVDP in the Esax group was significantly higher than that in the Control group (**Table 1**). The LVEDP values were approximately 90 mmHg higher than the baseline levels at the end of ischemia; however, the differences between the LVEDP values of the two groups were not statistically significant. The LVEDP values decreased gradually during reperfusion, ranging between 30 and 40 mmHg at the end of reperfusion. Statistically significant differences were observed between the values of the two groups (**Fig. 2B**). The differences between the temporal recovery of coronary flow (ml/g wt/min) in the two groups did not reach significance (**Fig. 3**).

Myocardial injury

The reduction in total troponin T leakage (ng/g wt) in the Esax group was significantly higher than that in the Control group (**Fig. 4**).

Inhibition of NOS

The LVDP recovery of the Esax group was approximately 60% of that in the pre-ischemic condition. The L-NAME + Esax group similarly recovered but lower than those of the Esax group (**Fig. 5A**). The recovery of coronary flow in the Esax group was significantly higher than that in the L-NAME + Esax group (**Fig. 5B**).

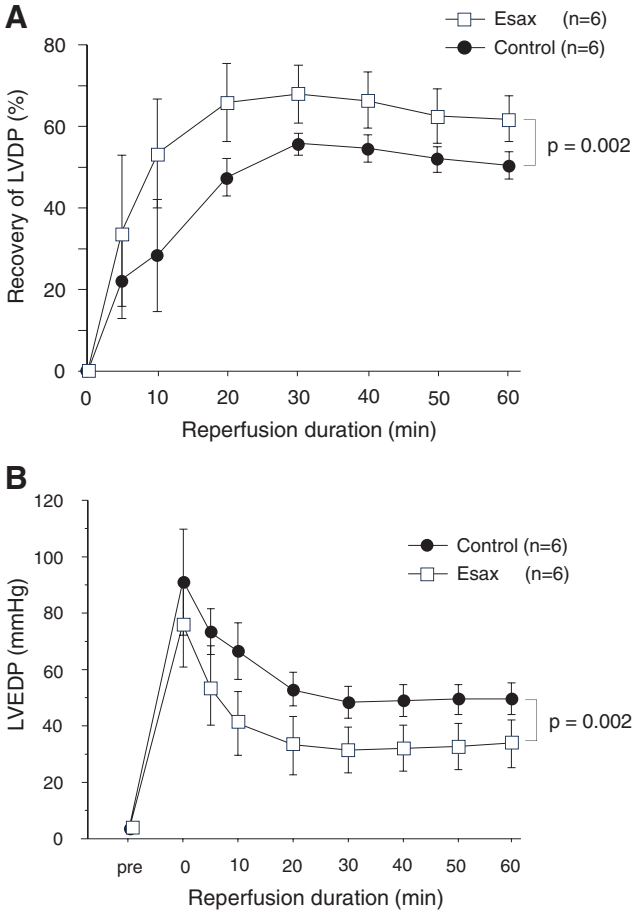

Fig. 2 (**A**) Recovery of the LVDP (expressed as a percentage of the baseline value) and (**B**) changes in the LVEDP (expressed as values during reperfusion). Filled circle: Control; open square: Esax. STH2: St. Thomas' Hospital No. 2 solution; LVDP: left ventricular developed pressure; LVEDP: left ventricular end-diastolic pressure

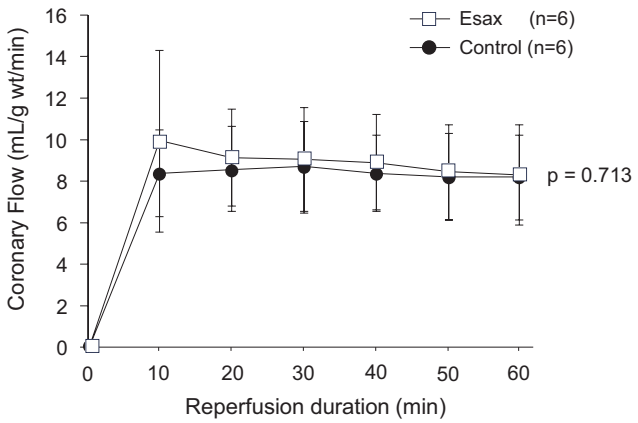

Fig. 3 Coronary flow (ml/g wt/min) during 60 min of reperfusion. (i) Control group: STH2 treatment only, (ii) Esax group: infusion with 0.1 μmol/L esaxerenone-dissolved KHB for 10 min before ischemia. STH2: St. Thomas' Hospital No. 2 solution; KHB: Krebs–Henseleit bicarbonate

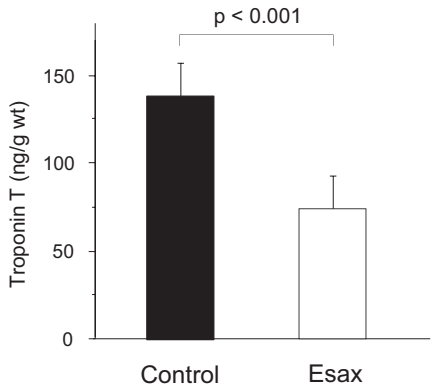

Fig. 4 Total troponin T leakage (expressed as ng/g wt) during 60 min of reperfusion. (i) Control group: 60-min reperfusion with KHB (STH2 treatment only) (ii) Esax group: infusion with 0.1 μmol/L esaxerenone dissolved in KHB for 10 min before ischemia. STH2: St. Thomas' Hospital No. 2 solution; KHB: Krebs–Henseleit bicarbonate

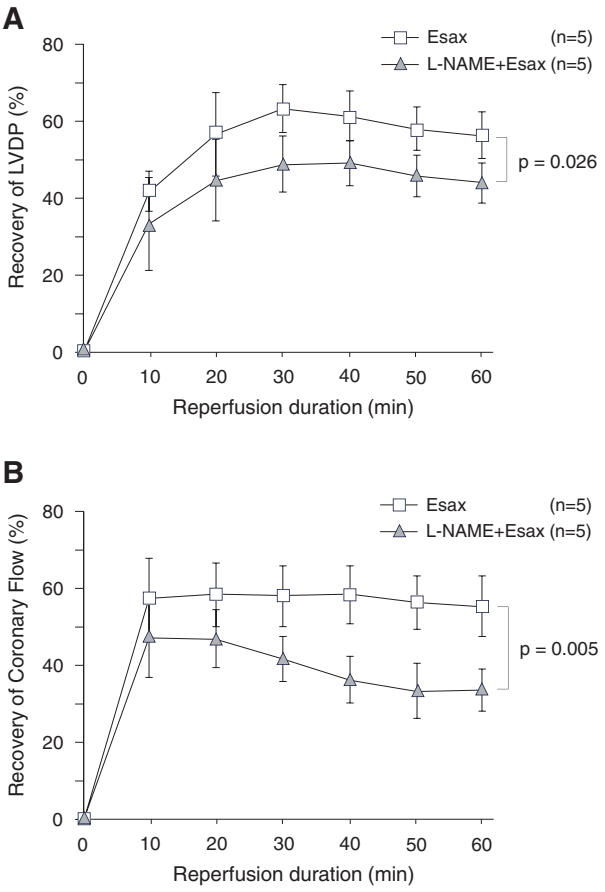

Fig. 5 (**A**) Recovery of the LVDP (expressed as a percentage of the baseline value) and (**B**) Recovery of the CF (expressed as a percentage of the baseline value). Open square: Esax; gray triangle: L-NAME + Esax. STH2: St. Thomas' Hospital No. 2 solution; L-NAME: N(x)-nitro-L-arginine monomethyl ester; LVDP: left ventricular developed pressure; CF: coronary flow

Discussion

This study evaluated the cardioprotective efficacy of esaxerenone, a non-steroidal MRB, after cardioplegic arrest in isolated rat hearts. To the best of our knowledge, this is the first study to demonstrate the additive cardioprotective effect of MRB in conjunction with STH2. The findings of the present study suggest that the administration of esaxerenone enhances the myocardial protective effects of hyperkalemic cardioplegia during cardiac surgery.

The cardioprotective effects of MRBs against infarctions in several species have been reported in previous studies. Chai et al. reported that the pre-ischemic administration of spironolactone reduced ischemic injury in isolated rat hearts.[12] Similarly, the administration of eplerenone improves the condition of rat hearts after ischemia and reperfusion by reducing the size of the infarct and enhancing left ventricular pressure recovery.[13] This protective effect could be related to the blockade of the proarrhythmogenic actions of aldosterone and/or the aldosterone-induced increase in oxygen radical synthesis. Chai et al. suggested that aldosterone was still present in the isolated perfused rat Langendorff heart.[13] Steroidal MRBs, such as spironolactone and eplerenone, reduce mortality[5]; however, steroidal MRBs are not prescribed routinely in clinical settings owing to concerns regarding the risk of hyperkalemia and hormonal side effects.

The discovery of non-steroidal MRBs has opened an avenue in cardiorenal disease therapy.[6] Rahman et al. revealed that animals fed a high-salt diet (HSD) developed cardiac dysfunction, as evidenced by the reduction in stroke volume, ejection fraction, and cardiac output.[14] In addition, the administration of esaxerenone decreased the worsening of cardiac dysfunction and induced a significant reduction in systolic blood pressure. Furthermore, the administration of esaxerenone reduced cardiac remodeling and fibrosis in HSD-fed Dahl salt-sensitive (DSS) rats. The administration of esaxerenone also led to a significant reduction in the mRNA expression of NADPH oxidase in HSD-fed DSS rats and the malondialdehyde levels in the cardiac tissues of DSS rats.[14]

Mineralocorticoid receptors are involved in inflammation, remodeling, fibrosis, and endothelial dysfunction.[8] MR-triggered Sodium/Hydrogen Exchanger 1 activation after stretch leads to intracellular Na^+ and Ca^{2+} overload, calcineurin activation, and pathological cardiac hypertrophy.[15] Furthermore, aldosterone inhibits the activation of nitric oxide (NO) synthase,[16] which is a potential cause of vascular endothelial damage. NO inhibits factors, such as cytokinesis, vascular smooth muscle proliferation, and endothelial injury, that are known to cause myocardial damage. The administration of spironolactone has been reported to increase NO bioactivity and ameliorate vascular endothelial dysfunction.[17] Matsumoto et al. reported that the administration of esaxerenone improves endothelial function by increasing endothelium-derived hyperpolarizing signaling and suppressing endothelium-derived contracting factor signaling in diabetic rats.[18] The administration of esaxerenone also reduced diabetes-induced endothelial dysfunction in C57B/6 mice by improving the phosphorylation of endothelial NO synthase.[19]

The development of several cardioplegic solutions has led to an improvement in myocardial protection during cardiac surgery. Extracellular hyperkalemic solutions are commonly used in clinical practice; however, exposure of the coronary artery endothelium to hyperkalemic cardiac arrest and ischemia–reperfusion injury adversely affects coronary endothelial function.[20–22] Repeated administration of hyperkalemic cardioplegia can result in a significant reduction in NO release from the coronary vasculature during cardiac arrest.[23] Moreover, the downregulation of endothelial NO synthase caused by hyperkalemic cardioplegia–reperfusion can also impair the release of NO.[24] Therefore, further additives for intraoperative myocardial protection or alternatives to hyperkalemic solutions must be developed. A previous study demonstrated that esaxerenone preserves endothelium-dependent vasorelaxation after ischemia–reperfusion, which is consistent with the findings of the present study.[9]

Limitations

The current study has certain limitations. An isolated heart preparation without collateral circulation was used in this study. Myocardial ischemic disease is a multifactorial disease characterized by a spectrum of injuries that affect myocardial protection. The hearts used in this study were obtained from healthy rats under normal feeding conditions; thus, it is possible that the protective effect of esaxerenone may differ in older hearts and jeopardized hearts with ischemic injury. Lastly, a short duration of ischemia was adopted in this experimental study despite such hearts requiring prolonged periods of ischemia in clinical settings.

Conclusion

The addition of esaxerenone, a non-steroidal MRB, enhanced the myocardial protective effect exerted by STH2 in isolated rat hearts. This effect was attenuated by a NOS inhibitor. The administration of esaxerenone before cardioplegic arrest may attenuate myocardial injury and improve surgical outcomes in clinical settings.

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Declarations

Ethics approval and consent to participate

The Animal Ethics Committee of the Nippon Medical School approved this study (no.: 2020-079).

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Data availability statement

Data are available on reasonable request to the corresponding author.

Author contributions

Masahiro Fujii: Conceptualization and methodology, formal analysis of the study, and drafting of the manuscript. Hiromasa Yamashita: Funding acquisition and investigation. Yasuhiro Kawase: Analysis of arrhythmia in the study; critical revision of the work for important intellectual content. Ryuzo Bessho: Validation and critical review of the work for important intellectual content. Yosuke Ishii: Supervision: Critically revising the work for important intellectual content.

Disclosure statement

None declared.

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