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Early Aerobic Exercise Combined with Hydrogen-Rich Saline as Preconditioning Protects Myocardial Injury Induced by Acute Myocardial Infarction in Rats

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Abstract It has been reported that hydrogen-rich saline (HRS) water reduces oxidative stress, and early aerobic exercise (eAE) acts an efficient exercise preconditioning (EP) against cardiac I/R injury. However, whether early aerobic exercise combined with hydrogen-rich saline (eAE-HRS) water can more effectively protect myocardial damage induced by acute myocardial infarction (MI) is still unknown. This study was aimed to evaluate the effect of eAE-HRS in preventing MI-induced myocardial damage and explore the possible underlying mechanisms. After Sprague-Dawley (SD) rats were given a intragastric administration of HRS (1.6 ppm) at a dosage of 10 mL/kg weight daily for 3 weeks and/or the SD rats were performed a eAE program with 3 weeks running training, the left anterior descending coronary artery was ligated to induce MI. We assessed the effects of eAE-HRS on myocardial injury and oxidative damage in the MI model of rats and detected the effects of eAE-HRS on the expressions of cardiac OGG1 and Tom40, Tom20, and Tim23. The eAE-HRS increased significantly left ventricular systolic pressure, reduced left ventricular end-diastolic pressure, and potentiated + dp/dt_{max}, $-dp/dt_{max}$, heart coefficient and pH after MI injury. The eAE-HRS reduced MI-induced CK-MB level, c-Tnl level, h-FABP level, infarct size. The eAE-HRS enhanced MI-induced levels of the superoxide dismutase and total antioxidant capacity, attenuated MI-induced levels of malondialdehyde and catalase. The eAE-HRS increased expressions of OGG1, Tom20 and Tim23 proteins after MI injury, but not Tom40. The eAE-HRS has the potential to be a novel precautionary measure to protect myocardial injury after MI via partially regulating expressions of antioxidant-related proteins and mitochondrial-associated proteins.

Keywords Aerobic exercise · Hydrogen-rich saline · Cardioprotective · Myocardial infarction

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Abbreviation

CAT	catalase
CK-MB	creatine kinase isoenzyme
cTn-I	cardiac troponin I
eAE	early aerobic exercise
eAE-HRS	early aerobic exercise combined with hydrogen-rich saline
ECG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GSH-PX	glutathione peroxidase
H&E	hematoxylin and eosin
h-FABP	heart type fatty acid binding protein
HRP	horseradish peroxidase
HRS	hydrogen-rich saline
LVEDP	left ventricular end-diastolic pressure
LVSP	left ventricular systolic pressure
HC	heart coefficient
MDA	malondialdehyde
MI	myocardial infarction
mtDNA	mitochondrial DNA
OGG1	8-oxoguanine DNA glycosylase
T-AOC	total antioxidant capacity
Tim23	translocase of inner mitochondrial membrane 23
Tom20	translocase of outer membrane 20
Tom40	translocase of the outer mitochondrial membrane 40
T-SOD	superoxide dismutase
TTC	2,3,5-triphenyl tetrazolium chloride
RNS	reactive nitrogen species
ROS	reactive oxygen species
8-OHdG	8-hydroxydeoxyguanosine

Introduction

Acute myocardial infarction (MI) is one of the major causes of death in the world; MI induced myocardial necrosis and apoptosis, interstitial fibrosis, non-infarct area compensatory changes, and the development of ventricular remodeling ultimately leading to heart failure is at the background of a high mortality rate [26, 27]. Epidemiological studies suggest that physically active individuals have a 30–50% lower risk of developing cardiovascular disease than do sedentary persons [4, 14]. With the development of medical technology, there has been more and more clinical treatment for myocardial infarction, but this has also brought a high risk and expensive problems. How to effectively protect the myocardium against ischemic risk and prevent the decrease of cardiac function caused by acute MI has a great significance.

Oxidative stress is involved in MI injury, and mitochondria electron transport is an enzymatic source of oxygen radical generation [11]. After MI, the increased oxygen radical produced in the mitochondria not only damages mitochondrial DNA (mtDNA) which leads to

mitochondrial dysfunction, but also induces cardiac function failure [29]. Mitochondrial function also requires import of proteins from the cytosol via the translocase of the outer and inner membrane (TOM and TIM complexes) [22]. Recent evidence has indicated that generation of reactive oxygen species (ROS) increases after ischemia reperfusion [35]. High levels of lipid peroxides occur during lipid peroxidation with polyunsaturated fatty acids in the biological membrane which destroy cell membranes and subcellular membrane structures, leading to cellular injury and death [10, 12].

Physical activity has been proposed to be a good intervention to prevent and improve cardiovascular diseases. Prospective epidemiological data has indicated that regular aerobic exercise can reduce the incidence of cardiovascular events [8]. And studies have shown that moderate exercise after MI can improve left ventricular remodeling and cardiac dysfunction, and moderate exercise can attenuate clinical symptoms of MI patients [23]. It has been reported that exercise preconditioning (EP) which is similar to ischemic preconditioning (IP) could reduce heart injury-after myocardial ischemia (MI) and ischemia reperfusion (I/R) injury. It may active antioxidant system and render the heart more tolerant to a subsequent ischemic injury [33]. Early exercise can strengthen physical fitness against some disadvantageous events, so daily and reasonable exercise is helpful for preventing MI.

Researchers have done a lot of experimental research on hydrogen since Ohsawa et al. found that hydrogen has antioxidant and antiapoptotic activity for cerebral ischemia reperfusion injury in 2007 [20]. They found that hydrogen plays an important role in many areas such as heart, brain, and liver; in atherosclerosis; and in other diseases. As a new type of effective antioxidant, hydrogen has a broad prospect in the prevention and treatment of the injury induced by MI.

We investigated whether or not the combination of eAE and HRS can protect myocardial injury, and what was regulatory mechanism. The primary goal of this study was to provide new thought for the prevention of the development and progression of myocardial infarction.

Materials and Methods

Animals and Grouping

Male Sprague-Dawley rats were purchased from Laboratory Animal Center of Xi'an Jiao Tong University (Animal quality certificate No. 08-004) at the age of 3 months and fed randomly with standard chow and water. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. All the rats were fed adaptively for 1 week, and then the animals were randomly divided into five experimental groups: Ctrl (normal control group, $n = 12$), SMI (sedentary + MI group, $n = 12$), HMI (hydrogen-rich saline + MI group, $n = 12$), EMI (early aerobic exercise + MI group, $n = 12$), HEMI (hydrogen-rich saline+ early aerobic exercise + MI group, $n = 12$).

eAE Protocol

Rats were allowed to exercise using a small animal treadmill (DSPT-202, Li Tai Technology, Hangzhou, China) with zero inclination. For the aerobic exercise program, the 3-week running training model was employed [33]. In detail, the adaptive training was executed with a

treadmill at a speed of 15 m/min for 20 min/day in the first week; incremental training was performed with a treadmill at a speed of 30 m/min for 30 min/day in the second week; and intermittent training was employed with a treadmill at a speed of 30 m/min for 60 min/day and there were three times rest periods during the running in the third week.

Preparation and Administration of HRS

HRS was produced by spraying the water with a high-pressure pump from the hydrogen gas tank at a pressure of 0.8 MPa. The final concentration of hydrogen gas in HRS was approximately 1.6 ppm. The saturated HRS was stored at 4 °C under atmospheric pressure and used within 1 week [19]. The HRS was placed into the stomach of the rats in a dosage of 10 mL/kg weight daily for 3 weeks. In addition, the rats undergoing training were administrated with the HRS 30 min before the start of the training.

MI Operation

After the training and administration, acute myocardial infarction operation was performed on the rats. All experimental procedures used in the study have previously been published in detail [30]. In brief, rats were sedated with intraperitoneal injection of 5% pentobarbital sodium (30 mg/kg); small animal respiratory mask which is pressure-controlled ventilated with O_2/N_2 [1:2(vol/vol)] was used to assist breathing. MI was produced by ligation of the left anterior descending coronary artery. The success of the operation was determined by electrocardiogram (ECG). The hemodynamic measurements were performed at 45 min after MI. Immediately after these measurements, rats were sacrificed; blood samples were collected into tubes, which were then centrifuged at 3500 rpm for 5 min; and serum samples were stored at − 80 °C until analysis. For analysis of pH in peripheral blood, the blood samples were collected under the sealed condition, and the pH was measured by the same pH electrode potentiometric method in the first affiliated hospital of Xi'an Jiaotong University (Shaanxi, China).

Electrocardiogram and Hemodynamic Measurements

ECG and invasive hemodynamic were performed as previously described [32]. After the mice were anesthetized with pentobarbital sodium (30 mg/kg), the lead II of ECG was recorded to monitor the success of the MI model by using RM6240 physiological signal acquisition system. Meanwhile, a transducer with pressure sensor was inserted right carotid artery into the left ventricle. LV systolic pressure (LVSP, mmHg), LV end-diastolic pressure (LVEDP, mmHg), maximal rate of rise (+ dp/dt max), and maximum rate of decline (− dp/dt max) indicators were measured and calculated by using Powerlab 8/30 (ML 870, AD Instruments, Castle Hill, Australia).

TTC Staining

For 2,3,5-triphenyl tetrazolium chloride (TTC) staining, the heart was transected with 2 mm thickness at 45 min after MI, and then the sections were incubated into 2% fresh TTC solution for 15 min under the conditions of protection from light. The images were collected with Nikon COOLPIX P900s camera.

Ultrastructural Observation

After washing, the fixed specimens were placed into 2% osmium tetroxide and then washed by phosphate buffer and dehydrated. Then, they were embedded in Epon 812 and cut into 50–60 nm thickness, then stained with uranyl acetate and lead citrate and examined by using electron microscopy (Model H-7650, Hitachi) [31].

Tests of Myocardial Injury Markers

Myocardial injury markers including creatine kinase isoenzyme (CK-MB), cardiac troponin I(cTn-I); heart-type fatty acid binding protein (h-FABP) in blood samples were tested by using the double antibody sandwich enzyme-linked immunosorbent assay (ELISA, R&D, USA).

Tests of Myocardial Oxidative Stress Markers

The myocardial tissue oxidative stress markers were collected using the methods as described by Adeneye et al. [1]. The methods described were used to determine the superoxide dismutase (T-SOD), malondialdehyde (MDA), glutathione peroxidase (GSH-PX), catalase (CAT), and total antioxidant capacity (T-AOC).

Western Blot Analysis

Total proteins were extracted using the tissue total protein extraction kit (Amresco, USA) from the myocardium of rats. All of the primary antibodies were purchased from Abcam (Abcam, UK). The proteins were separated by using SDS-PAGE followed by electrotransfer to an NC membrane; the membranes were probed using antibodies against OGG1 (1:500), Tom40 (1:500), Tom20 (1:500) and Tim23 (1:200), followed by a horseradish peroxidase (HRP)-conjugated second antibody. GAPDH (1:10000) (Abcam, UK) was used as internal reference. Bands were revealed with ECL reagent (Millipore, USA) and recorded on X-ray films (Kodak, China). The densitometry of each band was quantified by a gel imaging system and Quantity One 4.62 software (Bio-rad, USA) [39].

Statistical Analysis

Data are presented as means ± SEM. Statistical analysis was performed with SPSS13.0 software (IBM, USA). Statistical evaluation of the data was performed using one-way ANOVA and LSD for multiple comparisons. Significant differences are defined at a p value of < 0.05.

Results

The eAE-HRS Improves Haemodynamics of Rats Subjected to MI Injury

Compared with SMI, the indexes of hemodynamics in the HEMI group, such as LVSP and LVEDP, the maximal ascending rate of left ventricular pressure($+dp/dt_{max}$), the maximal descending rate of left ventricular pressure($-dp/dt_{max}$), HC, and pH, were all significantly

improved (Table 1). Moreover, the degree of improvement in HEMI group was weakly better than that in the HMI or EMI group. These results revealed that the combination of eAE and HRS effectively ameliorates the cardiac dysfunction of rats subjected to MI.

The eAE-HRS Attenuates Myocardial Injury against MI

Compared with SMI, the contents of CK-MB in serum were reduced in the HEMI group ($p < 0.01$, Fig. 1a), and the reduction in HEMI group was the most among the HMI, EMI, and HEMI groups. Similar results were obtained in the detection of contents of cTn-I and h-FABP. The contents of cTn-I and h-FABP in HEMI were significantly reduced compared with that in SMI ($p < 0.01$, $p < 0.01$), and the reduction in the HEMI group was the most among the HMI, EMI, and HEMI groups (Fig. 1b, c). These statistical data suggested that the administrations including eAE, HRS, and eAE-HRS all ameliorated myocardial injury against MI, especially eAE-HRS administration.

Compared with SMI, infarct size in HMI and HEMI were significantly reduced ($p < 0.05$, $p < 0.05$, Fig. 1d, e), suggesting that administration of HRS and eAE-HRS alleviated myocardial injury against MI.

Results of ultrastructural observation in the SMI group showed serious injury including incomplete sarcomere and its irregular arrangement, irregular arrangement of Z line, mitochondrial structure destruction, cristae disappearance, and mitochondrial vacuole-like shape increase, compared with that in Ctrl (Fig. 1f). Compared with SMI, these phenomena indicating myocardial ultrastructural injury in HMI, EMI, and HEMI have been restored. In HMI group, it displayed regular arrangement of sarcomere and Z line, slight swelling of partial mitochondria, but cristae clearness (Fig. 1f). In the EMI group, it displayed slight regular arrangement of sarcomere and Z line, little irregular arrangement of myofilaments, and slight swelling of mitochondria (Fig. 1f). However, in the HEMI group, most cardiomyocytes displayed normal structure, sarcomere and Z line regularly arranged and had no outspread phenomenon; the number of mitochondria increased; and cristae was clear (Fig. 1f). These results suggested that eAE, HRS, and eAE-HRS all could repair myocardial ultrastructural lesions against MI, especially eAE-HRS administration.

The eAE-HRS Increases Myocardial Antioxidation against MI

The imbalance between oxidation-antioxidation system causes massive productions of free radicals contributing to myocardial oxidative damage [9]. Compared with SMI group, the activities of T-SOD, GSH-PX, and T-AOC were increased in the HEMI group ($p < 0.01$; $p < 0.05$; $p < 0.05$), and the increase in HEMI group was the most among the HMI, EMI, and HEMI groups (Fig. 2a, c, e). The contents of MDA and CAT were obviously reduced in the HEMI group compared with that in the SMI group ($p < 0.01$, $p < 0.05$), and the reduction in the HEMI group was the most among the HMI, EMI, and HEMI groups (Fig. 2b, d). These data indicated that eAE and HRS, especially eAE-HRS, all improved myocardial antioxidation against MI injury.

The eAE-HRS Ameliorates Mitochondrial Dysfunction Against MI

Long-term production and accumulation of oxygen free radicals in the body can lead to a catastrophic recurrent mitochondrial DNA damage [29]. Therefore, repair of mitochondrial

Table 1 The indexes of haemodynamics of experimental rats subjected to MI injury

Groups	LVSP (mmHg)	LVEDP (mmHg)	+ dp/dtmax (mmHg/s)	− dp/dtmax (mmHg/s)	HC (mg/g)	pH (peripheral blood)
Ctrl	114.59 ± 9.29	5.43 ± 2.41	6497.87 ± 1183.10	4604.14 ± 1045.72	3.62 ± 0.47	7.38 ± 0.20
SMI	94.82 ± 17.68*	14.25 ± 6.94**	2620.62 ± 869.68**	2133.39 ± 438.14**	3.64 ± 0.15	7.93 ± 0.14**
HMI	110.17 ± 13.80	8.20 ± 1.47	3440.80 ± 740.74	4074.65 ± 1649.51	4.07 ± 0.24	7.44 ± 0.19
EMI	103.32 ± 8.70	9.69 ± 0.64	4596.49 ± 394.49	3544.06 ± 1380.95	3.81 ± 0.32	7.46 ± 0.16
HEMI	112.76 ± 7.70##	5.66 ± 0.96##	4919.81 ± 917.69##&	4188.20 ± 1707.68#	4.16 ± 0.16##	7.40 ± 0.19##

Values are mean ± SD; $n = 6$ rats for each group

Ctrl normal control group, *SMI* sedentary + MI group, *HMI* hydrogen-rich saline + MI group, *EMI* early aerobic exercise + MI group, *HEMI* hydrogen-rich saline+ early aerobic exercise + MI group

*$p < 0.05$; **$p < 0.01$ Ctrl vs SMI;# $p < 0.05$; ##$p < 0.01$ SMI vs HEMI; &$p < 0.05$ HMI vs HEMI

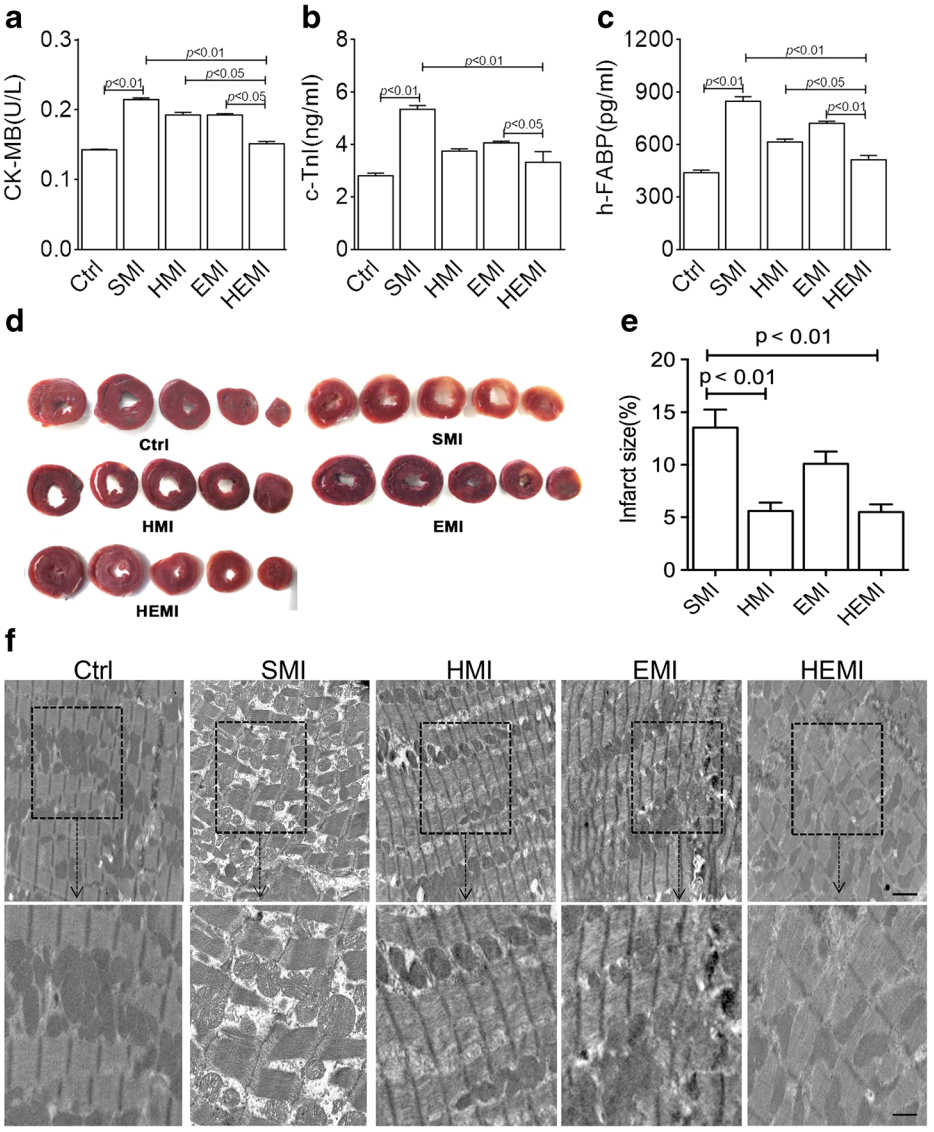

Fig. 1 The effect of eAE-HRS on myocardial injury against MI. **a** The content of serum CK-MB. **b** The content of serum cTn-I. **c** The content of serum h-FABP. Values are mean ± SD, $n = 6$ rats, for each group. **d** MI was stained by TTC staining; the heart of ischemic tissue is pale. **e** Infarct size. **f** The ultrastructure of cardiomyocytes was visualized by transmission electron microscope. Scale bar 2 μm. The lower image is an amplification of the upper black box with scale bar is 1 μm

function will contribute to the improvement of MI injury. Compared with SMI group, the expression of mtDNA repairase OGG1 was significantly increased in the HEMI group ($p < 0.01$), and the increase in the HEMI group was the most among the HMI, EMI, and HEMI groups (Fig. 3a, b). The expression of Tom40, which is the main component of transport complex in the mitochondrial outer membrane, had no significant difference among the five groups (Fig. 3a, c). Additionally, the expression of Tom20, which is the main

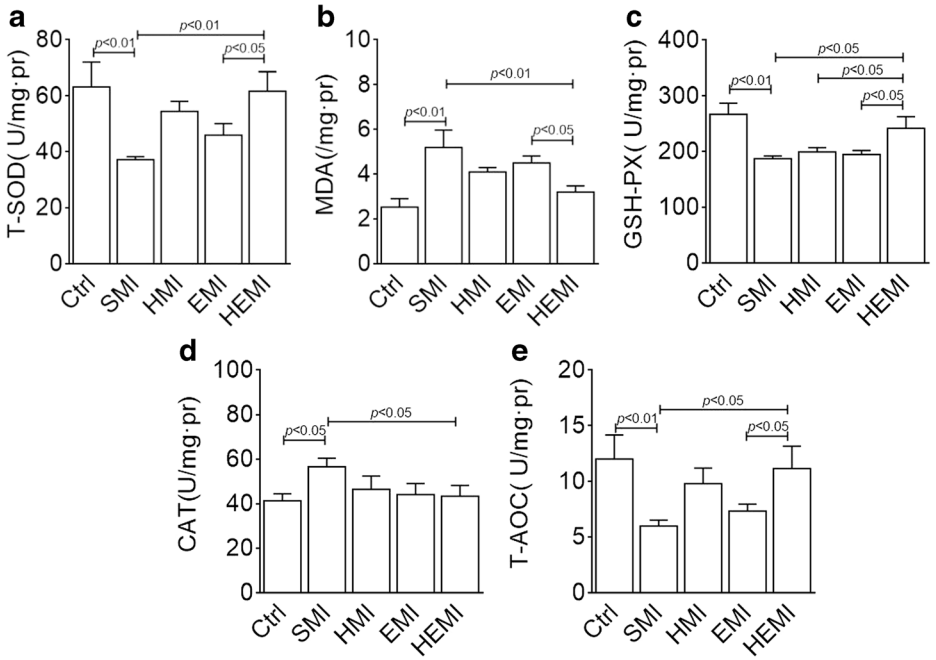

Fig. 2 The effect of eAE-HRS on myocardial antioxidation against MI. **a** The activity of T-SOD in the myocardium was measured using spectrophotometer. **b** The content of MDA in the myocardium was detected using spectrophotometer. **c** The content of GSH-PX in the myocardium was measured using spectrophotometer. **d** The activity of CAT in the myocardium was tested using spectrophotometer. **e** The activity of T-AOC in the myocardium was measured using spectrophotometer. Values are mean ± SD, $n = 6$ rats for each group

component of transport complex in the mitochondrial inner membrane, was also increased in the HEMI group compared with the SMI group ($p < 0.01$), and the increase had no significant difference among the HMI, EMI, and HEMI groups (Fig. 3a, d). Compared with SMI, the expression of Tim23, which often interacts with many precursor proteins, was enhanced in the HEMI group ($p < 0.05$), and the enhancement in the HEMI group was the most among the HMI, EMI, and HEMI groups (Fig. 3a, e). These results revealed that administrations of eAE and HRS, especially eAE-HRS, promoted potentially mitochondrial DNA repair mechanisms and mitochondrial biosynthesis after MI injury.

Discussion

In this study, rats were administered with eAE, HRS, and eAE-HRS as preconditioning, respectively. Myocardial injury (MI) was then induced in the rats by ligaturing the left descending anterior coronary artery to explore the effect of the preconditioning on myocardial injury. Our results indicate that eAE-HRS modulates systolic and diastolic function of heart to prevent MI injury. Furthermore, administration of eAE, HRS, and especially eAE-HRS was able to ameliorate MI injury and repaired myocardial mitochondrial function by regulating mitochondrial repair- and biosynthesis-related proteins. Additionally, eAE-HRS enhanced myocardial antioxidation against MI injury.

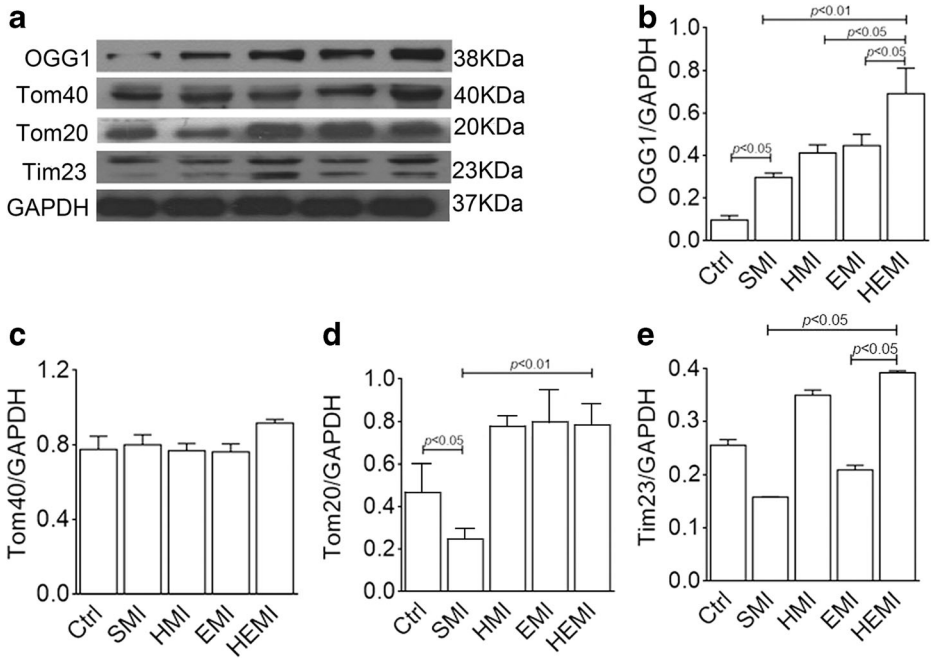

Fig. 3 The effect of eAE-HRS on mitochondrial protein transport function against MI. **a** The expression of OGG1 and mitochondrial transport protein in the myocardium after MI injury was tested by using western blot. **b** The expression of OGG1 protein in the myocardium after MI injury was detected by using western blot. **c** The expression of Tom40 protein in the myocardium after MI injury was detected by using western blot. **d** The expression of Tom20 protein in the myocardium after MI injury was measured by using western blot. **e** The expression of Tim23 protein in the myocardium after MI injury was tested by using western blot; Values are mean ± SD, $n = 6$ rats for each group

The eAE-HRS increased significantly LVSP, reduced LVEDP, and potentiated $+dp/dt_{max}$, $-dp/dt_{max}$, and heart coefficient after MI injury. Consistent with previous studies, HRS decreases destruction and degradation of aortic elastic fibers [6], indicating a positive effect of HRS on the alteration of arterial vascular wall elasticity. Additionally, HRS can significantly improve heart functional parameters after myocardial injury [28]. Therefore, it is possible HRS increases blood flow to the heart by increasing the elasticity of arterial blood vessels. It is well known that eAE increases the quantity and quality of muscle fibers [36]. So, eAE-HRS can alleviate myocardial injury which is likely associated with not only enhancement of muscle fibers but also repair of aortic elastic fibers.

After acute myocardial injury, cardiomyocytes undergo either apoptosis or necrosis, with associated cell membrane damage. As a result, small-molecule proteins, such as CK-MB, cTn-I, and h-FABP, can be released into the peripheral blood, resulting in their increase in peripheral blood [3, 21, 34]. In our study, eAE-HRS caused a reduction in the levels of CK-MB, cTn-I, and h-FABP after MI, indicating reduction of release of these small molecule proteins, thus contributing to the protection of myocardial cellular membrane integrality to ameliorate myocardial injury. Lei et al. previously used isoproterenol to induce MI in rats. The MI group was pretreated with physiological saline i.p., whereas the HRS group was pretreated with hydrogen-rich saline (5, 7.5, and 10 mL/kg body weight i.p.) before administration of isoproterenol. The results showed that infarct size and CK-MB activity reduced in HRS group

compared with saline-alone group. Due to the several experimental groups in our study, pretreatment of MI group with saline was not performed. However, the above-mentioned studies have shown that saline alone may not provide significant effects on myocardial injury.

To explore the mechanism of cardioprotective effect of eAE-HRS, we determine the association between myocardial antioxidation and mitochondrial function. After acute MI in rats, myocardial ischemia and hypoxia produced a large number of free radicals, reactive oxygen species (ROS) [15], and reactive nitrogen species (RNS), leading to myocardial oxidative damage. Among the ROS, •OH and $ONOO^-$ are much more reactive and would aggressively damage cellular macromolecules, including nucleic acids, proteins, and lipids [24]. Therefore, protecting and enhancing cellular antioxidant ability contributes to fend against myocardial injury. Previous studies have also proposed that HRS induces cardioprotection against myocardial injury in rats possibly through selectively reducing the hydroxyl radical and removing the most cytotoxicity of reactive oxygen species [18, 28]. HRS protects heart against ischemia/reperfusion by suppressing oxidative stress [7, 13, 38], including reducing myocardium MDA and 8-hydroxydeoxyguanosine (8-OHdG) concentration [28]. But HRS alone is not sufficient for improving myocardial injury. It is also known that the moderate aerobic exercise also weakly improves myocardial antioxidation and alleviates myocardial injury. However, exhaustive aerobic exercise can induce oxidative stress [2]. So, we used both HRS and eAE to effectively improve myocardial antioxidation and ameliorate myocardial injury of rats. While combination of exercise with other antioxidants, such as vitamin E and vitamin C, had no effect on MDA level and weakly reduced 8-OHdG level [5], indicating exercise may be more suitable to combine with HRS than vitamin E and vitamin C to regulate myocardial antioxidation. Therefore, eAE-HRS was used to enhance myocardial antioxidation against MI injury.

Some reports have indicated that eAE protects the heart against ischemic injury by preventing caspase-3-related mitochondria apoptotic signaling pathway [36]. Furthermore, it was able to reverse the mitochondrial dysfunction, as determined by improved mitochondrial membrane integrity and energy metabolism. Our study has confirmed that eAE-HRS is able to enhance regular arrangement of sarcomere and Z line in myocardial ultrastructure to protect mitochondrial structure and function after MI. Also, a recent study indicates that the protein expression of Tom40 and Tim23 did not increase in the myocardium of exercising compared to non-exercising pigs, while the protein expression of Tom20 was increased [25]. However, in our study, eAE-HRS increased expressions of both Tom20 and Tim23 proteins after MI injury, but not Tom40, indicating that it could enhance mitochondrial biosynthesis, therefore having a strong relationship with the protection of MI injury. Apart from reduction of mitochondrial protein synthesis, damage of mitochondrial DNA repair and replication mechanism is among the causes of mitochondrial dysfunction. Exercise training is able to decrease ROS production, attenuate DNA oxidative damage, and increase the activity of DNA repair processes [16] to aid the removal of oxidatively damaged proteins. The eAE is known to increase expression of mtDNA repairase OGG1 after MI injury, suggesting a role in DNA repair function of cardiomyocytes to protect MI injury. Furthermore, mitochondria are important sites for ROS production which is considered as primary cause of cardiac trauma. On the other hand, accumulation of massive ROS in the mitochondria can cause oxidative damage to mtDNA and further induce mtDNA mutations. Therefore, reduction of ROS production contributes to the amelioration of mitochondrial dysfunction. HRS protects the heart against ischemia/reperfusion injury by reducing mitochondrial ROS production [17, 37], which may be related to increase of OGG1 expression by HRS. Thus, eAE combined with HRS not only improves

mitochondrial structure but also effectively ameliorates mitochondrial dysfunction, including increasing mitochondrial biosynthesis and DNA repair function against MI injury. Our study also had limitations. It is not powered to detect mitochondrial membrane potential and ATP production which generally reflect the mitochondrial energy production and the function of mitochondria. We only proved the effect of combination of hydrogen water and exercise interventions against injury induced by MI by reducing oxidative stress and improving the function of mitochondrial channel proteins.

Conclusions

In summary, eAE-HRS as a preconditioning intervention protects hearts against MI in rats and attenuated the MI-induced myocardial injury in the early phase of cardioprotection. eAE-HRS increased myocardial antioxidation and improved mitochondrial structure. The import machinery of mitochondria and DNA repair function may play an important role in the cardioprotective mechanism afforded by eAE-HRS.

Author Contributions TZJ conceived of the study. FR and CMX performed the experiments and collected and analyzed all data. TZJ, FR, and WXD prepared the manuscript, and all the authors edited the manuscript. All the authors contributed to the writing of the manuscript.

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Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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