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Epoxyeicosatrienoic acid analog EET-B attenuates post-myocardial infarction remodeling in spontaneously hypertensive rats

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Abstract

Epoxyeicosatrienoic acids (EETs) and their synthetic analogs have cardiovascular protective effects. Here, we investigated the action of a novel EET analog EET-B on the progression of post-myocardial infarction (MI) heart failure in spontaneously hypertensive rats (SHR). Adult male SHR were divided into vehicle- and EET-B (10 mg/kg/day; p.o., 9 weeks)-treated groups. After two weeks of treatment, rats were subjected to 30-min left coronary artery occlusion or sham operation. Systolic blood pressure and echocardiography measurements were performed at the beginning of study, 4 days before, and 7 weeks after MI. At the end of the study, tissue samples were collected for histological and biochemical analyses. We demonstrated that EET-B treatment did not affect blood pressure and cardiac parameters in SHR prior to MI. MI was induced and cardiac parameters were measured 7 weeks later. Fractional shortening (FS) was decreased to 18.4±1.0% in vehicle-treated MI rats compared with corresponding sham (30.6±1.0%) 7 weeks following MI induction. In infarcted SHR hearts, EET-B treatment improved FS (23.7±0.7%),

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AUTHOR CONTRIBUTION

JN primarily conceived and designed the study, performed experiments, analyzed and interpreted the data and wrote the manuscript. JDI, GJG and MAHK designed the study and wrote the manuscript. JDI, JRF and WBC designed and synthesized EET-B. MAHK, MC, JH, AK, LS, ŠŠ, VO, MG, DS, and FK were involved in performing the experiments, data collection, analysis and interpretation, contributed to the intellectual content and editing of the manuscript, and approved its final version.

COMPETING INTERESTS

The Author(s) declare that there are no competing interests associated with the manuscript.

markedly increased heme oxygenase-1 immunopositivity in cardiomyocytes and reduced cardiac inflammation and fibrosis (by 13% and 19%, respectively). In conclusion, these findings suggest that EET analog EET-B has beneficial therapeutic actions to reduce cardiac remodeling in SHR subjected to MI.

Keywords

spontaneously hypertensive rat; myocardial infarction; blood pressure; congestive heart failure; epoxyeicosatrienoic acid

INTRODUCTION

Congestive heart failure (CHF) is a leading cause of morbidity and mortality that created major healthcare and economic burden in the Western world. In most instances, CHF is the irreversible and final consequence of a variety of etiologies; however, a predominant cause is coronary heart disease [1]. Several factors such as hypertension, diabetes mellitus or obesity contribute to CHF development and progression as well as organ damage to lung and kidneys. Although considerable progress has been made in CHF management, novel therapeutic strategies are required.

Epoxyeicosatrienoic acids (EETs), cytochrome P450 epoxygenase metabolites of arachidonic acid, represent a promising pharmacological approach in cardiovascular disease prevention. A large body of evidence indicates that EETs are important paracrine agents with robust organ protective actions. EETs prevent various cardiovascular diseases, due to their vasodilator, anti-hypertensive, anti-inflammatory and other beneficial biological actions [2,3]. The main limitation for EETs as a therapeutic application is their short half-life due to EETs being quickly converted to biologically inactive dihydroxyeicosatrienoic acids (DHETs) by the enzyme soluble epoxide hydrolase (sEH). Considering these facts, it is conceivable that any effective EETs therapeutic utilization for cardiovascular diseases can only be achieved by two major approaches.

The first approach is based on pharmacological blockade of sEH to reduce the degradation of EETs to DHETs. Previously, we and others established that the acute administration of sEH inhibitors improved cardiac ischemic tolerance in normotensive and hypertensive animals [4–7]. Moreover, it was determined that chronic pharmacological intervention with sEH inhibitors reduced myocardial hypertrophy and fibrosis development and progression, decreased inflammation and improved heart function post-myocardial infarction (MI) [8–11].

A second approach involves the use of agonistic EET analogs with markedly longer (hours) half-life. The primary advantage of this strategy is that EET analogs are biologically more stable than endogenously produced EETs. Hence, several EET analogs have been developed and demonstrated powerful protective actions against several cardiovascular diseases [12–20]. Here, cardioprotective effects of a novel, stable and orally active agonistic 14,15-EET analog EET-B [15] was examined in spontaneously hypertensive rats (SHR), a pre-clinical rodent model of human essential hypertension [21]. SHR were subjected to MI and the

effects of continuous EET-B treatment before and after MI on post-ischemic left ventricular (LV) function, myocardial fibrosis and inflammation were analyzed. As EET-based therapies can attenuate the progression of CHF by mechanisms involving activation of heme oxygenase-1 (HO-1) [22]. HO-1 immunopositivity in viable myocytes of the ischemic myocardium was also determined. Our findings demonstrate that EET-B treatment has beneficial actions on the SHR heart following MI.

METHODS

Animal groups and experimental protocol

The EET analog EET-B [*N*-isopropyl-*N*-(5-((2-pivalamidobenzo[*d*]thiazol-4-yl)oxy)pentyl)heptanamide] (Figure 1A) was designed and synthesized in the laboratory of John R. Falck. Pharmacokinetic studies analyzing oral administration of EET-B predicted its half-life at 10-15 hrs [23,24]. The EET-B dose was based on our previously published studies that determined the dose-response for vascular reactivity and showed antihypertensive and renal protective effects in salt-sensitive and angiotensin II-dependent hypertension [15,16,25].

The Medical College of Wisconsin Institutional Animal Care and Use Committee according to the National Institutes of Health Guidelines for Care and Use of Laboratory Animals approved all animal studies. Ten-week old male spontaneously hypertensive rats (SHR) were purchased from Charles River Laboratories (Wilmington, MA, USA). Animals were housed in the Biomedical Resource Center at Medical College of Wisconsin with a 12/12h light-dark cycle and free access to tap water and rodent chow. SHR were divided into two groups. The first group received vehicle (0.05% ethanol and 0.1% PEG-400), while the second group received EET-B (10 mg/kg/day; p.o.) from 2 weeks before MI till 7 weeks thereafter.

SHR were weighed and systolic blood pressure (SBP) was measured by tail-cuff plethysmography (IITC Life Science Inc., Woodland Hills, CA) at the beginning of study, 4 days before and 2, 4 and 7 weeks after MI (Figure 1B). Rats were trained for blood pressure measurement at the same time of the day (9 – 12 am) for 3 days before every intervention.

Echocardiographic assessment of cardiac function

Echocardiography was carried out to determine function of the left ventricle (LV) by an ultrasound-based noninvasive technique (Transthoracic Echocardiography, Visual Sonics Vevo 770 with RMV 710B probe, Toronto, Canada). Animals were anesthetized with 2% isoflurane (Forane; Abbott Laboratories, Queenborough, UK) mixed with room air. Prior to starting the treatment protocol, echocardiography measurements were performed at baseline and then 4 days before and 7 wks after myocardial infarction (MI) or sham operation in anesthetized rats (Figure 1B). Diastolic and systolic dimensions of left ventricle (LV) were measured during echocardiography evaluation including anterior and posterior wall thickness (AWTd, PWTd, AWTs, PWTs) and LV cavity diameter (LVDd, LVDs). From these dimensions, the following functional echocardiography parameters were derived: fractional shortening (FS) using the formula $(LVDd-LVDs)/LVDd*100$, systolic thickening of anterior wall (AWT) from the equation $(AWTs-AWTd)/AWTd*100$, and relative wall thickness from the equation $(AWTd-PWTd)/LVDd*100$.

Myocardial ischemia

After the second echocardiography evaluation (before MI), Vehicle- or EET-B-treated SHR were divided into four groups: i) Sham-Vehicle, ii) MI-Vehicle, iii) Sham-EET-B, iv) MI-EET-B. Anesthetized (sodium pentobarbital, 60 mg/kg i.p.) open-chest rats ventilated with room air (1.2 ml/kg) were subjected to 30 min of left anterior descending (LAD) coronary artery occlusion or sham surgery as described previously [26]. At the start of reperfusion, the chest was closed, air was removed from the thorax and spontaneously breathing animals recovering from anesthesia were housed in separate cages and received analgesia (Buprenorphine, 0.5 – 1 mg/kg s.c.) followed for another two days. The incidence and severity of ventricular arrhythmias during the 30-min ischemic insult were assessed according to the Lambeth Convention as previously described [27]. Premature ventricular complexes (PVCs) occurring as singles, salvos or tachycardia (VT, a run of 4 or more consecutive PVCs) were counted separately. The incidence and duration of life-threatening ventricular tachyarrhythmias, i.e. VT and fibrillation (VF), were also evaluated. VF lasting more than 2 min was considered as sustained (VFs). Rats exhibiting VFs were excluded from further evaluation.

Histopathological studies

The day after the final echocardiography measurements, rats were euthanized by cervical dislocation and hearts were collected for histological analysis. Hearts were excised, washed with Tyrode's solution, perfusion-fixed and stored in 4 % paraformaldehyde for 2 days at 4° C. Hearts were cut perpendicularly to the long axis at the largest circumference and embedded in paraffin. Tissue sections were cut into 4 μ m slices for use in histology protocols. Formalin-fixed and paraffin-embedded tissue slices were de-paraffinized, rehydrated and stained with Hematoxylin-Eosin or Picro-Sirus Red staining for gross histopathological examination and determination of collagen positive fibrotic areas in the heart. Tissue sections were also immunostained with anti-HO-1 (1:1000, Abcam, Cambridge, UK). Horseradish peroxidase-conjugated goat anti-rabbit secondary antibody (1:200; Jackson ImmunoResearch Laboratories, west Grove, PA, USA) was used for development with avidin-biotinylated HRP complex (Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA, USA) followed by counterstaining with hematoxylin. Stained sections were visualized by light microscopy at 400 \times magnification and digital images of the stained sections were taken for analysis using Nikon NIS Elements Software (Nikon Instruments Inc., Melville, NY, USA). Collagen content and HO-1 expression were determined by quantification of Picro-Sirus Red stained and HO-1-positive areas in viable cardiomyocytes, respectively (10-15 images for each heart sections) by two experienced observers in a blinded fashion.

Statistical analysis

The results are expressed as means \pm SEM. Unpaired Student's t-test or one-way ANOVA and subsequent Student-Newman-Keuls test were used for comparison of differences in normally distributed variables between groups. Not normally distributed data (arrhythmias) were expressed as median (range). Differences in the number of premature ventricular complexes between the groups were compared by Mann-Whitney test. The incidence of

tachycardia and fibrillation and mortality were examined by Fischer's exact test. Differences were considered statistically significant when $P < 0.05$.

RESULTS

EET-B treatment did not affect blood pressure and body weight

At the beginning of the study, the experimental groups did not differ in SBP and body weight (BW; Figure 2A,B). In both MI groups, a significant reduction in BW was evident one week after MI. However, from the second week post-MI, weight gain occurred in the groups subjected to ischemia, and by the end of the study protocol BW was similar among all groups (Figure 2A). Surprisingly, EET-B pretreatment to SHR prior to MI had no effect on SBP (Figure 2B). MI significantly reduced SBP by 35 – 45 mmHg seven weeks following MI due to heart failure in both vehicle- and EET-B-treated groups compared to corresponding sham-operated rats.

EET-B treatment reduced ischemic arrhythmias and early post-MI mortality

Sustained VF occurred in one vehicle-treated MI SHR. Four vehicle-treated and one EET-B-treated SHR died in the early post-ischemic period (within 48 hrs. after MI). Therefore, ischemic and early reperfusion mortality reached 29.4% and 9.1%, respectively, in vehicle- and EET-B-treated SHR and did not increase during 7 week follow up post-MI period (Figure 2C). Interestingly, EET-B-treated SHR exhibited lower incidence and severity of ischemic ventricular arrhythmias compared to vehicle-treated SHR but the differences did not reach statistical significance (Table 1).

EET-B treatment attenuated the progression of post-MI heart failure

As summarized in Figure 3 and Table 2, LV systolic function and geometry assessed by echocardiography were not altered by vehicle or EET-B treatment prior to MI. A significant increase in systolic and diastolic LV diameters were found in sham vehicle- and EET-B-treated controls at the end of study. The observed dilation of the LV chamber in both sham-operated groups reflected a significantly lower FS (by 16.3% and 14.2%, respectively) compared to values prior to surgery (Figure 3A). In the SHR group subjected to MI and vehicle-treated, both FS and AWT were markedly decreased to $18.4 \pm 1.0\%$ and $24.5 \pm 4.4\%$, respectively, compared to age-matched sham-operated controls that averaged $30.6 \pm 1.0\%$ and $57.5 \pm 2.6\%$, respectively. EET-B treatment significantly attenuated MI-induced reductions in FS and AWT to $23.7 \pm 0.7\%$ and $39.1 \pm 3.6\%$, respectively (Figure 3).

The relative lungs weight significantly increased by 75.4% in the Mi-vehicle group compared with corresponding sham-operated controls. In MI-EET-B group, the lungs weight increase was significantly less pronounced (31.1%) suggesting that EET-B treatment attenuated the progression of CHF-induced lung edema (Table 3). With respect to heart weight (HW), MI increased HW/BW ratio by 10% and 13%, respectively, compared to corresponding sham-operated groups (Table 3).

EET-B treatment reduced post-MI fibrosis and inflammation

In the vehicle-treated SHR ischemic area, collagen fraction as determined by Picro-Sirius Red staining and monocytes/macrophages infiltration as determined from the presence of CD68 positive cells reached $42.8\pm 1.5\%$ and $2.18\pm 0.11\%$, respectively (Figure 4). EET-B treatment significantly reduced myocardial fibrosis and CD68 positive area to $37.2\pm 1.9\%$ and $1.77\pm 0.11\%$, respectively. In non-ischemic septum of vehicle- or EET-B-treated SHR subjected to MI or Sham operation, collagen content as well as monocytes/macrophages infiltration did not differ among the groups (Figure 4).

EET-B treatment increased HO-1 immunopositivity in viable LV cardiomyocytes post-MI

In both sham-operated SHR groups (vehicle- and EET-B-treated), HO-1 immunopositivity in cardiomyocytes was low and averaged $0.20\pm 0.08\%$ and $0.25\pm 0.11\%$, respectively (Figure 5A). MI increased HO-1 cell immunopositivity in non-ischemic septa in both vehicle- and EET-B-treated SHR (Figure 5). This finding suggests that post-MI cardiac remodeling could activate HO-1-mediated cell signaling. Moreover, EET-B treatment markedly increased HO-1 immunopositivity in viable cardiomyocytes in the infarcted area to $46.0\pm 4.4\%$ (Figure 5).

DISCUSSION

The main finding of the present study is that the orally active EET analog, EET-B reduced post-MI mortality and systolic dysfunction progression in SHR. Cardio-protective actions for EET-B were associated with diminished CHF-induced lung edema, reduced myocardial fibrosis, decreased monocytes/macrophages infiltration in the ischemic area, and increased HO-1 immunopositivity in viable cardiomyocytes after MI. Taken together, we demonstrate that EET-B attenuates CHF progression without altering blood pressure in SHR with established hypertension.

It is well known that EETs possess multiple biological functions including vasodilation, anti-inflammatory, anti-fibrotic, and also affects lipid metabolism and insulin sensitivity [2,9,28–30]. EETs have been demonstrated to contribute to neovascularization by promoting angiogenesis [31,32]. In the heart, the acute administration of EETs or sEH inhibitors prior to ischemia and at the start of reperfusion increased cardiac ischemic tolerance in several animal species and experimental settings [4,6,7,33,34]. Recently, cardio-protective actions for acute administration of EET analogs against ischemia/reperfusion injury have been determined. Indeed, UA-8, a dual acting compound possessing EET mimetic and sEH inhibitory properties, improved cardiac ischemic tolerance in isolated mouse hearts [35]. Likewise, EET-A, another orally active EET analog with similar protective potential to EET-B [36], reduced myocardial infarction in normotensive Sprague-Dawley rats [12]. Finally, in a recent study we revealed a potent infarct size-limiting effect of acute EET-B administration given at the start of reperfusion in normotensive rats [14]. We further demonstrated that EET-B provided cardio-protection comparable to native 14,15-EET, and its protective action was completely abolished by co-administration with 14,15-EEZE, the selective antagonist of 14,15-EET [14]. These findings clearly demonstrated that EET-B is an effective agonistic 14,15-EET analog.

Previous experimental studies demonstrated that chronic treatment with sEH inhibitors results in increased EET levels and diminishes the development of LV hypertrophy due to pressure or volume overload [19,37–42]. We reported that two-week treatment with orally active EET-analogs attenuate hypertension-induced organ injury by reducing inflammation, oxidative stress, and endoplasmic reticulum stress [15,16]. One orally active EET analog, EET-A reduced the incidence of life-threatening ischemic arrhythmias in transgenic *Ren-2* rats with angiotensin II-dependent hypertension [19] which is consistent with the anti-arrhythmic action of EET-B observed in the present study.

Interestingly, the cardio-protective effects for EET-B in the present study were independent of blood pressure independent. The hypertension severity before MI and the reduction of SBP due to heart failure were similar in vehicle- and EET-B-treated SHR. Blood pressure-independent kidney and myocardial protective actions for EET-B have been reported also in Dahl salt-sensitive (SS) hypertensive rats [15]. The inability for EET-B to exert a blood pressure independent action in Dahl SS rats and in SHR post-MI could be related to the fact that, unlike native EETs, EET-B lacks a natriuretic effect [15]. However, based on previous evidence for blood pressure independent organ protective actions with sEH inhibitor administration in SHR [43], we cannot exclude that SHR, a model of essential hypertension, or other hypertensive rat models with a different genetic background could be less sensitive to anti-hypertensive EET-based therapy than that consistently observed in angiotensin II-dependent hypertension [16,19,33,44]. In any case, our findings of the present study clearly indicate that EET-B had blood pressure independent actions to improve heart function in SHR with CHF.

Coronary artery disease is the main cause of CHF development [1] and has a very poor prognosis [45]. Therefore, determining pathophysiological mechanisms underlying post-MI cardiac remodeling and development of new therapeutic approaches are needed. In the present study, we investigated cardioprotective ability of EET-B. However, it should be noted that the published findings are not completely clear with respect to EET cardio-protective actions on the progression of heart dysfunction in humans after MI. Monti et al. [40] reported lower plasma 14,15-DHET levels and decreased *EPHX2*, the gene coding sEH, in myocardial biopsies harvested at the time of cardiac surgery in patients with severe CHF. This finding was interpreted as an adaptive transcriptional process aimed at maintaining high EET levels. Zhang et al. [46] observed attenuation of 14,15-DHET plasma levels in cardio-renal disease patients with decreased cardiac function and increased risk of adverse myocardial events. Schuck et al. [47] demonstrated an inverse association between coronary artery disease severity and the plasma 14,15-EET/DHET ratio, a biomarker for sEH activity. Finally, it has been reported that an sEH inhibitor increased EET levels in endothelial progenitor cells (important for vasculogenesis and re-endothelization), that are collected from patients following acute MI [29].

Unlike human, animal studies on the cardio-protective potential for EET-based therapy in failing hearts after MI are more consistent. It has been shown that NUDSA, an earlier generation EET analog, improved cardiac function in mice after MI [18]. Moreover, an attenuation of post-MI LV dysfunction progression has been reported in rats and mice treated with various sEH inhibitors [8,10,11,37,48]. In line with these earlier findings, we

demonstrated that EET-B treatment (started two weeks before MI and continued till seven weeks after MI) attenuated post-MI LV systolic dysfunction and adverse changes of ventricular geometry in the present study. This EET-B treatment prior to ischemia in SHR could have additional effects on acute cardiac ischemic tolerance as suggested by the reduction of life-threatening arrhythmias [19]. In addition, the improved post-MI LV function is more likely attributable to EET-B beneficial actions during CHF progression as it was previously demonstrated for other EET-based pharmacological interventions [8,10,11,18,37,48]. Finally, in a recent work we demonstrated that sEH inhibitor c-AUCB and EET analog EET-A, given alone or in combination at reperfusion attenuated the progression of post-MI LV systolic dysfunction only in Sprague-Dawley but not in hypertensive *Ren-2* transgenic rats in spite of blood pressure reduction [19,49]. Moreover, these findings support previous findings indicating that the protective action of EET analogs against various cardiovascular diseases is independent of their antihypertensive actions.

The effect of EET-based therapy on the progression of CHF-associated etiologies other than ischemic heart disease is more inconsistent. Indeed, it has been reported that sEH inhibitors can reduce [11,42] or unchanged [50] the development of cardiac hypertrophy and diminish adverse cardiac remodeling in normotensive mice and rats subjected to pressure overload. Similarly, sEH inhibitors did not alter LV contractility in normotensive and hypertensive rats subjected to CHF induced by volume overload [51–53].

In the current study, EET-B treatment in SHR subjected to MI decreased cardiac fibrosis that resulted in improved FS and AWT compared to vehicle-treated SHR seven weeks following MI. Pathological remodeling and cardiac fibrosis are strongly associated with inflammation that contributes to CHF progression. Earlier studies in rodents clearly demonstrated protective effects for EET-based therapy in the cardiac fibrosis prevention after MI [8,10,11,18,48] and in hearts subjected to pressure overload [9,11,54,55]. All these findings support the idea that increased endogenous EET levels, as well as, EET analogs provide beneficial anti-fibrotic and anti-remodeling actions in the injured myocardium. It should be noted that among the approaches to increase endogenous EET levels and/or increase EET bioavailability by sEH inhibition, only pharmacological sEH inhibition seems to be effective. Indeed, sEH gene deletion (*EPHX2*^{-/-}) did not show any positive effect in an experimental setting of pathological remodeling and cardiac fibrosis after angiotensin II treatment [9], and, unexpectedly, increased mortality in mice due to cardiac arrest [56].

Another potential mechanism for EET-B treatment to attenuate CHF progression in SHR could be through anti-inflammatory actions. Previous studies revealed that sEH inhibitors reduce myocardial inflammation after MI [8,9]. Most importantly, we showed marked anti-inflammatory actions of EET-B associated with its robust kidney protective ability in rat models of salt-sensitive [15] and angiotensin-II dependent hypertension [44]. In this study, we demonstrated monocytes/macrophage infiltration into the ischemic area in vehicle-treated SHR with CHF, and it was reduced by EET-B treatment. Taken together, in post-MI SHR, EET-B mediated improvement of LV systolic function accompanied by reduction in cardiac fibrosis and inflammation.

EETs ability to interact with HO-1 is another apparent mechanism that has been attributed to organ protective action [57,58]. We examined role of HO-1 in EET-B cardio-protective actions in Mi-induced CHF in SHR. Our findings revealed that EET-B increased HO-1 level in viable LV myocytes of SHR subjected to MI. This finding supports previous experimental findings that EET induction of HO-1 is an essential event in protecting myocardium against acute ischemia/reperfusion injury and the progression to post-ischemic CHF [57,59]. Cao et al. [18] showed that EET analog NUDSA increased HO-1 protein level which was associated with improved LV systolic function and reduced collagen fraction in the scar of post-MI mouse hearts, and co-treatment with an HO activity inhibitor reversed this effect. In SHR, pharmacological HO activation attenuates the progression of post-MI LV dysfunction [60] and reduces cardiac inflammation [61]. Most importantly, another orally active EET analog EET-A prevented the development of obesity-induced cardiomyopathy by enhancing HO-1 signaling in mice [58]. Overall, our findings demonstrate that HO-1 is a contributing factor for EET-B mediated reduction of post-MI LV systolic dysfunction and CHF progression in SHR.

In summary, the present study examined the cardio-protective potential of a novel orally active EET analog EET-B in SHR with Mi-induced CHF. EET-B demonstrated cardio-protective actions with lower incidence and severity of ischemic ventricular arrhythmias, reduced CHF-induced lung edema, LV systolic dysfunction, myocardial fibrosis and inflammation in MI-induced CHF model. Our data also suggest that HO-1 signaling can mediate EET-B cardio-protective actions in SHR with Mi-induced CHF.

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ABBREVIATIONS

AWTd	diastolic anterior wall thickness
AWTs	systolic anterior wall thickness
AWT	systolic thickening of anterior wall
bpm	beats per minute
BW	body weight
CHF	congestive heart failure
DHETs	dihydroxyeicosatrienoic acids
14,15-DHET	14,15-dihydroxyeicosatrienoic acid

ECHO	echocardiography
EETs	epoxyeicosatrienoic acids
14,15-EET	14,15-epoxyeicosatrienoic acid
EF	ejection fraction
FS	fractional shortening
HO-1	heme oxygenase-1
HR	heart rate
LAD	left anterior descending coronary artery
LV	left ventricle
LVDd	diastolic left ventricle diameter
LVDs	systolic left ventricle diameter
MI	myocardial infarction
PVCs	premature ventricle complexes
PWTd	diastolic posterior wall thickness
PWTs	systolic posterior wall thickness
RWT	relative wall thickness
SBP	systolic blood pressure
sEH	soluble epoxide hydrolase
SHR	spontaneously hypertensive rats
SS	salt-sensitive
VF	ventricular fibrillation
VT	ventricular tachycardia.

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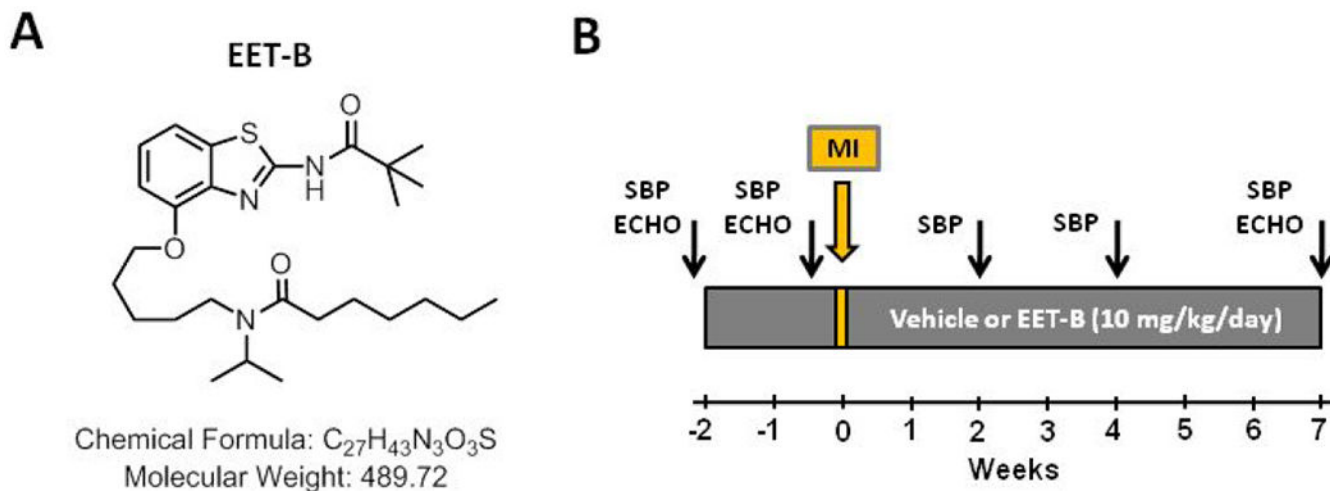
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Perspectives

- A novel EET analog EET-B improved cardiac functions independent of blood pressure in SHR with MI induced CHF.
- EET-B beneficial actions to improve LV systolic function in SHR with CHF is associated with increased cardiomyocyte HO-1 levels and reductions in cardiac fibrosis and inflammation.
- EET analog has beneficial cardiac actions and therapeutic potential for CHF.

**Figure 1.**

Structure of EET-B [*N*-isopropyl-*N*-(5-((2-pivalamidobenzo[*d*]thiazol-4-yl)oxy)pentyl)heptanamide] (**A**). The design of the experiments analyzing the cardio-protective action of EET-B in rat hearts (**B**). Systolic blood pressure (SBP) and echocardiography (ECHO) assessment of left ventricular function and geometry were performed at the start of the study, before MI and during 7 weeks of reperfusion. See Materials and Methods for the detailed description.

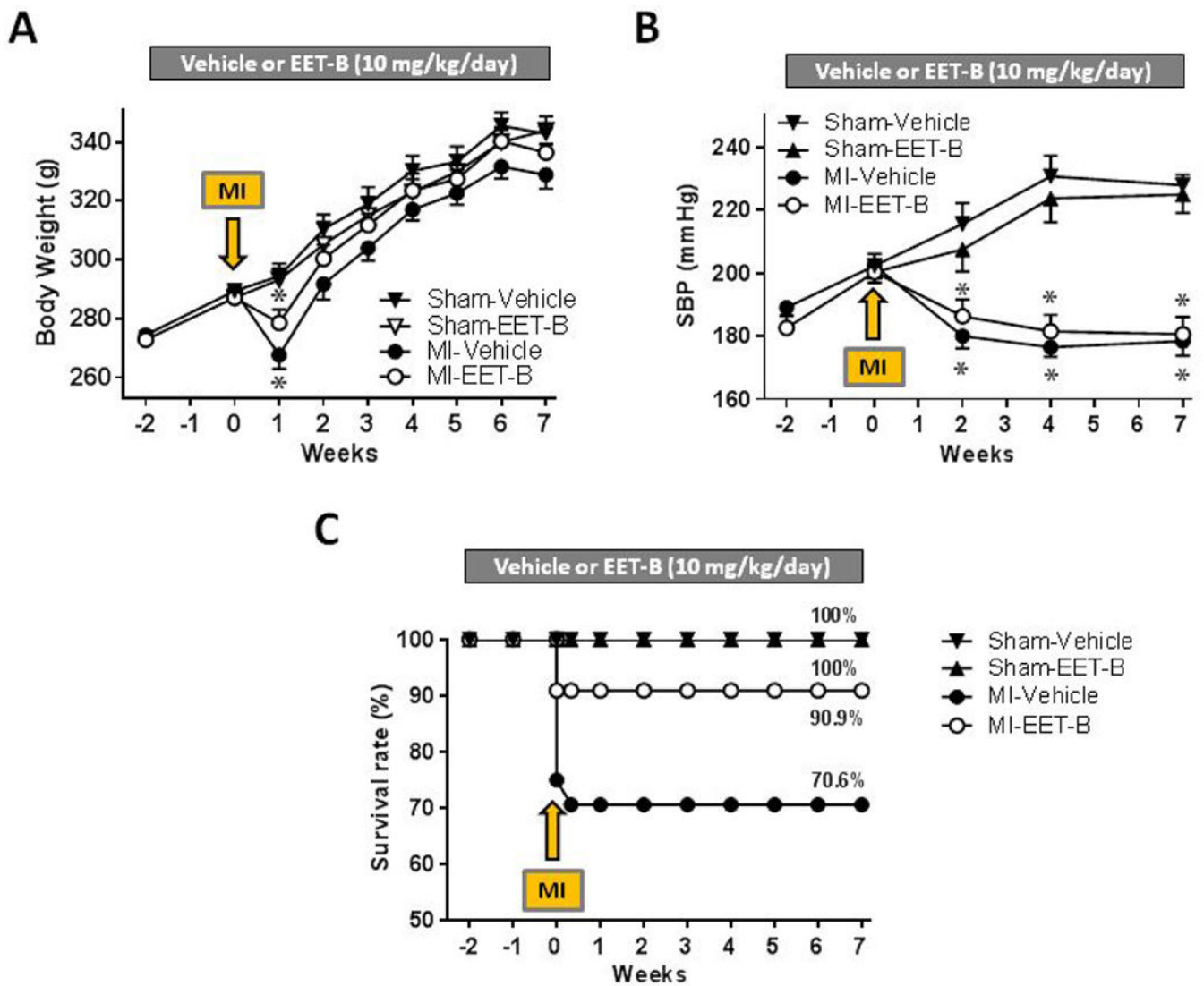


Figure 2. Body weight (A), systolic blood pressure (SBP) (B), and survival rate (C) in vehicle- and EET-B-treated SHR subjected to myocardial infarction (MI) and Sham-operated groups. Rats were treated by vehicle or EET-B (10 mg/kg/day) from two weeks before till seven weeks after MI. Values are means \pm SEM from 6-12 rats in each group. * P <0.05 vs. corresponding Sham-operated group.

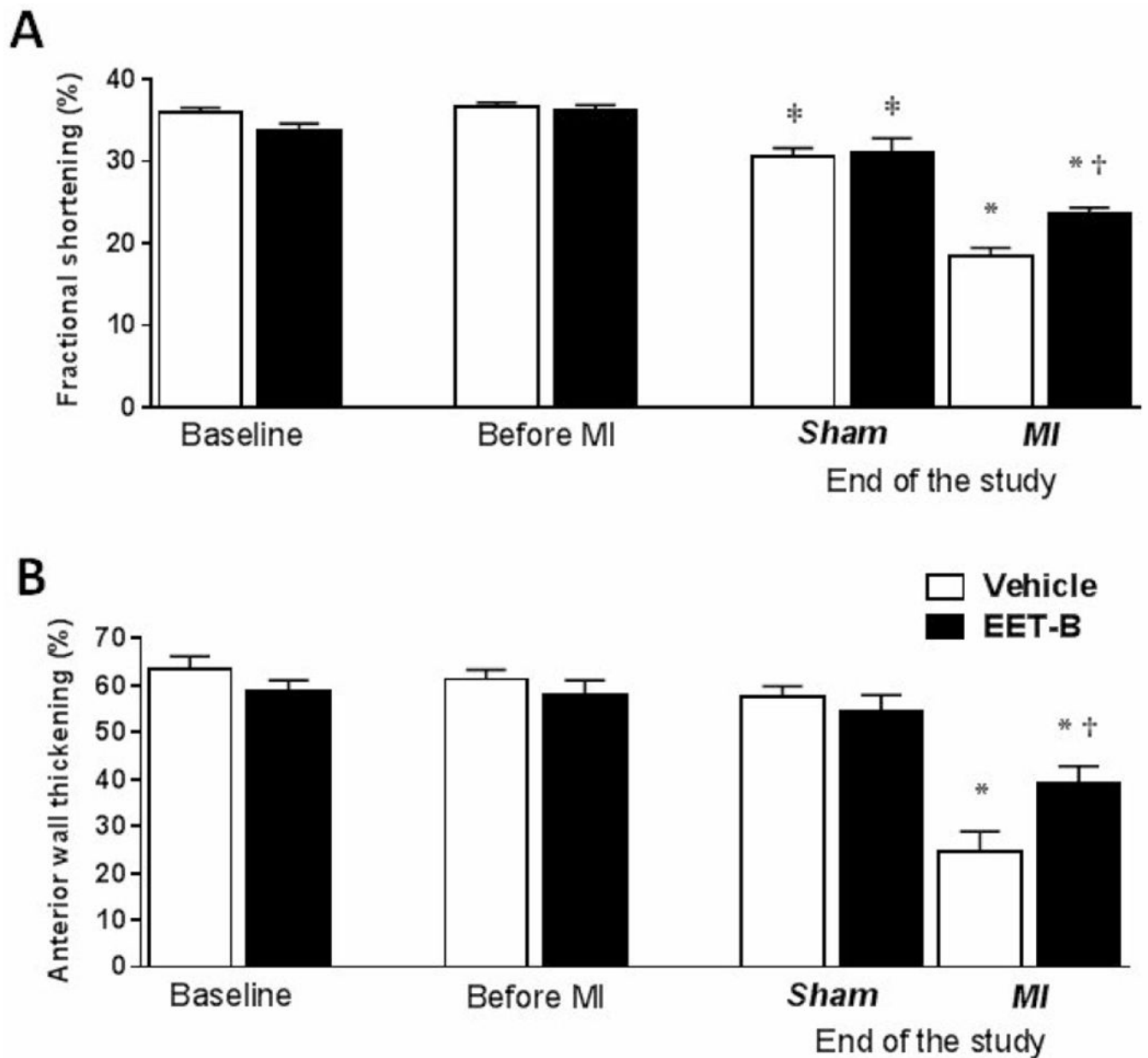


Figure 3.

Echocardiography assessment of left ventricular fractional shortening (**A**) and the anterior wall systolic thickening (**B**) in vehicle- and EET-B-treated SHR subjected to myocardial infarction (MI) and Sham-operated groups at the start of study (Baseline), after 10 days of treatment (Before MI) and 7 wks after MI (End of the study). Rats were treated by vehicle or EET-B from two weeks before till seven weeks after MI. Values are means \pm SEM from 6-12 rats in each group. * P <0.05 vs. corresponding Sham-operated group; † P <0.05 vs. MI-Vehicle group; ‡ P <0.05 vs. corresponding Before MI group.

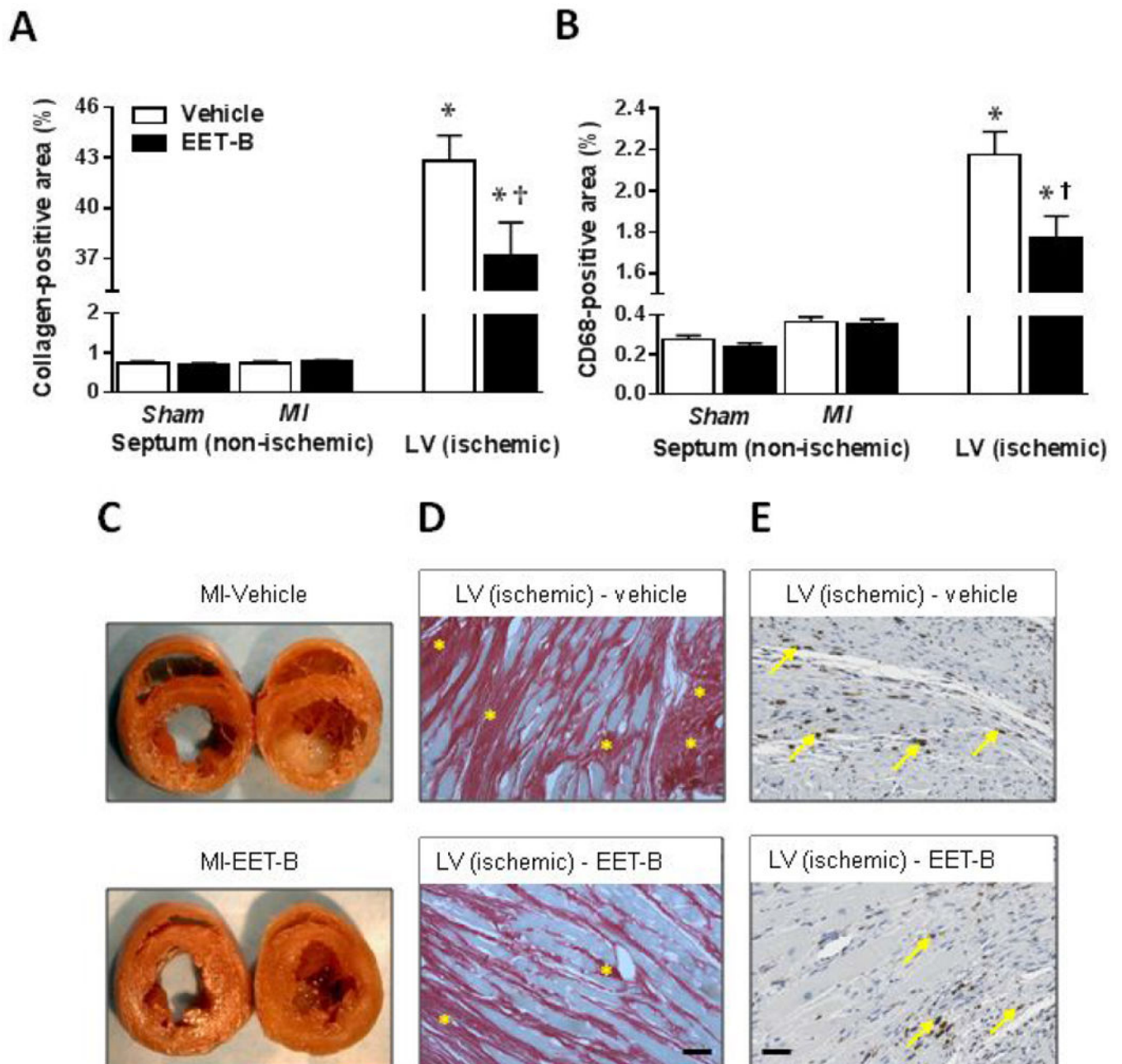


Figure 4.

Quantification of myocardial collagen-positive area (**A**) and CD68-positive area (**B**) assessed in non-ischemic septum, and ischemic area of left ventricle (LV) of vehicle- and EET-B-treated SHR subjected to myocardial infarction (MI) and Sham-operated groups.

Macroscopic images of heart cross-sections (**C**), photomicrographs of Picro-Sirius Red staining of collagen positive areas (carmine red marked by stars) (**D**), and immunohistochemical staining depicting macrophage/monocyte (CD68-positive; dark brown dots marked by arrows) (**E**). Rats were treated by vehicle or EET-B from two weeks before

till seven weeks after MI. Scale bars represent 50 μm . Values are means \pm SEM from 6-10 rats in each group. * $P < 0.05$ vs. Sham-Vehicle group. † $P < 0.05$ vs. MI-Vehicle group.

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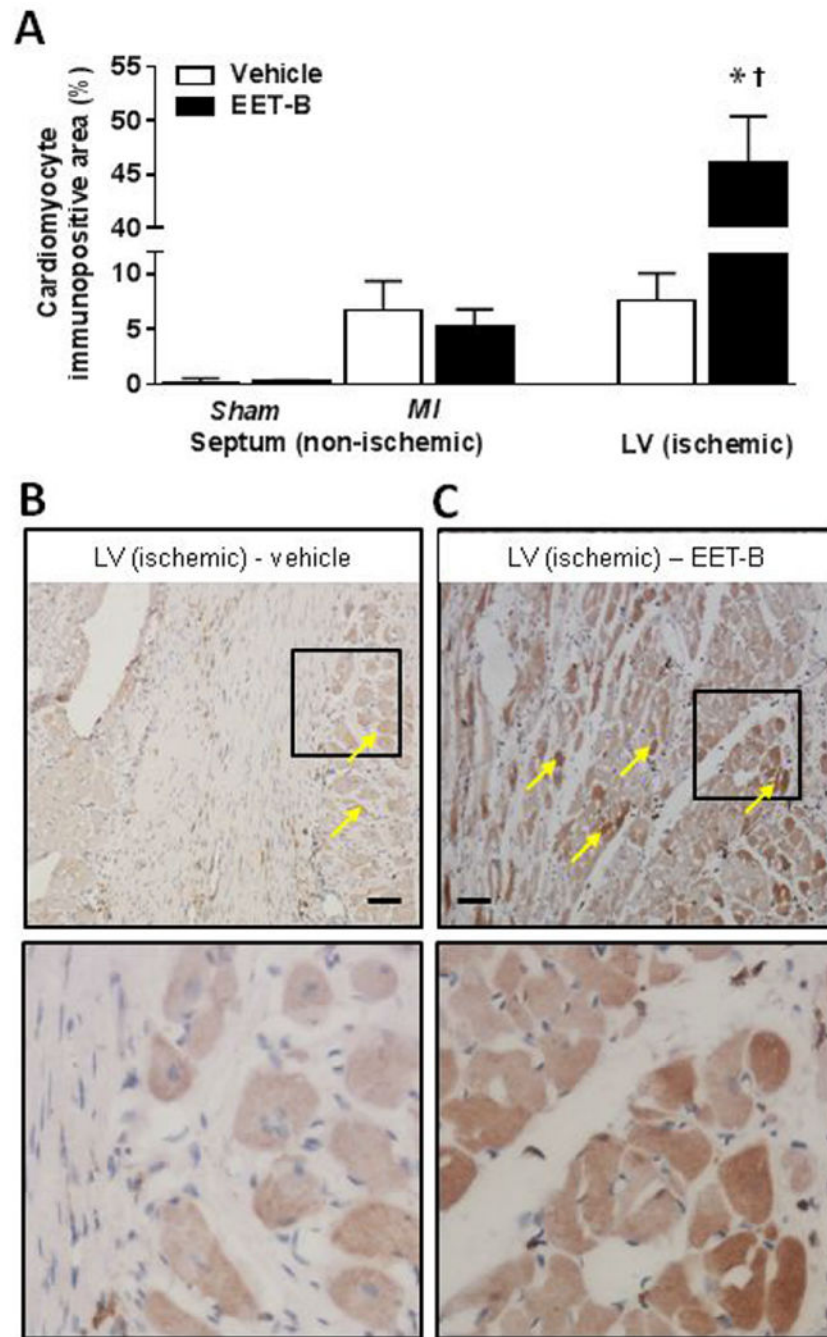


Figure 5. Quantification of heme oxygenase-1 (HO-1) immunopositivity in viable cardiomyocytes assessed in non-ischemic septum, and ischemic area of left ventricle (LV) of vehicle- and EET-B-treated SHR subjected to myocardial infarction (MI) and sham-operated groups (A), photomicrographs of immunohistochemical staining depicting HO-1 in ischemic area of LV (marked by arrows) in vehicle- (B) and EET-B-treated (C) rats. The lower pictures show the square areas in higher magnification. Rats were treated by vehicle or EET-B from two weeks

before till seven weeks after MI. Scale bars represent 75 μm . Values are means \pm SEM from 6-10 rats in each group. * $P < 0.05$ vs. Sham-Vehicle group. † $P < 0.05$ vs. MI-Vehicle group.

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Premature ventricle complexes (PVCs) occurring as singles, salvos and ventricular tachycardia (VT), incidence of VT and ventricular fibrillation (VF) and duration of tachyarrhythmias (VT+VF) during 30-min of coronary artery occlusion in vehicle- and EET-B-treated hearts of SHR.

Table 1

Group	n	Number of arrhythmias			Incidence (%)		Duration of tachyarrhythmias (s)	
		Singles	Salvos	VT	PVCs	VT		VF (VFs)
Vehicle	12	246(250)	167(187)	1750(3538)	2074(3310)	100	61.5 (7.7)	154(304)
EET-B	10	178(338)	93(147)	1240(2585)	1511(2383)	100	77.0 (0)	142(276)

n, number of animals; VFs, sustained VF; values are median (range).

Echocardiographic parameters of left ventricle in vehicle- and EET-B-treated SHR subjected to myocardial infarction (MI) or sham operation before start of treatment (Baseline), after 10 days of treatment (Before MI) and 7 weeks after MI (End of the study).

Table 2

	Baseline		Before MI		End of the study			
	Vehicle	EET-B	Vehicle	EET-B	Sham-Vehicle	Sham-EET-B	MI-Vehicle	MI-EET-B
AWTd (mm)	1.78 ± 0.04	1.93 ± 0.06	1.88 ± 0.05	1.98 ± 0.06	1.88 ± 0.09	2.02 ± 0.07	1.85 ± 0.14	1.94 ± 0.18
LVDd (mm)	7.32 ± 0.07	7.11 ± 0.12	7.36 ± 0.07	7.32 ± 0.09	8.16 ± 0.03 [‡]	8.16 ± 0.09 [‡]	9.60 ± 0.23 [*]	9.49 ± 0.15 [*]
PWTd (mm)	1.83 ± 0.02	1.82 ± 0.05	1.91 ± 0.04	1.91 ± 0.05	1.99 ± 0.07	1.98 ± 0.04	1.94 ± 0.08	2.06 ± 0.10
AWTs (mm)	2.90 ± 0.05	2.94 ± 0.05	3.02 ± 0.06	3.11 ± 0.07	2.96 ± 0.09	3.12 ± 0.06	2.34 ± 0.23 [*]	2.72 ± 0.30
LVDs (mm)	4.67 ± 0.07	4.74 ± 0.12	4.67 ± 0.07	4.67 ± 0.11	5.67 ± 0.10 [‡]	5.77 ± 0.12 [‡]	7.85 ± 0.26 [*]	7.25 ± 0.15 ^{*‡}
PWTs (mm)	2.73 ± 0.04	2.66 ± 0.07	2.86 ± 0.06	2.85 ± 0.06	2.86 ± 0.06	2.87 ± 0.11	2.56 ± 0.07 [*]	2.81 ± 0.14
RWT (%)	49.4 ± 1.0	53.0 ± 1.5	51.6 ± 1.5	53.4 ± 1.6	47.4 ± 1.9	48.9 ± 1.1	40.1 ± 3.1 [*]	42.1 ± 2.2 [*]
HR (bpm)	338 ± 7	308 ± 8	339 ± 9	350 ± 5	355 ± 9.2	337 ± 7	318 ± 7	332 ± 11

AWTd, diastolic anterior wall thickness; LVDd, diastolic left ventricle diameter; PWTd, diastolic posterior wall thickness; AWTs, systolic anterior wall thickness; LVDs, systolic left ventricle diameter; PWTs, systolic posterior wall thickness; RWT, relative wall thickness; HR, heart rate; bpm, beats per minute. Values are means ± SEM from 6-10 rats in each group.

* $P < 0.05$ vs. corresponding Sham group;

[‡] $P < 0.05$ MI-EET-B vs. MI-Vehicle group.

[‡] $P < 0.05$ vs. corresponding Before MI group.

Table 3

Relative weights of lung and heart 7 wks after MI in vehicle- and EET-B-treated SHR subjected to myocardial infarction (MI) and sham-operated.

	Lungs/BW (mg/g)	Heart/BW (mg/g)
Sham-Vehicle	3.58 ± 0.14	3.57 ± 0.02
Sham-EET-B	3.67 ± 0.09	3.47 ± 0.08
MI-Vehicle	6.28 ± 0.49 [*]	3.94 ± 0.07 [*]
MI-EET-B	4.81 ± 0.49 ^{*†}	3.93 ± 0.12 [*]

Values are means ± SEM from 6-10 rats in each group.

^{*} $P < 0.05$ vs. corresponding Sham group;

[†] $P < 0.05$ MI-EET-B group vs. MI-Vehicle group.