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SHORT COMMUNICATION

Beneficial Effect of Continuous Normobaric Hypoxia on Ventricular Dilatation in Rats With Post-Infarction Heart Failure

J. HRDLIČKA[1,2], J. NECKÁŘ[1], F. PAPOUŠEK[1], J. VAŠINOVÁ[1], P. ALÁNOVÁ[1], F. KOLÁŘ[1]

[1]Institute of Physiology, Czech Academy of Sciences, Prague, Czech Republic, [2]Department of Physiology, Faculty of Science, Charles University in Prague, Prague, Czech Republic

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Summary

Adaptation to continuous normobaric hypoxia (CNH) protects the heart against ischemia/reperfusion injury but much less is known about its potential therapeutic effects. The aim of this study was to find out whether post-infarction exposure to CNH can attenuate the progression of heart failure. Ten-week-old male rats underwent myocardial infarction (MI) or sham operation. MI was induced by 60-min coronary artery occlusion. Seven days post-MI, the rats were randomly assigned to two groups: i) sedentary controls kept at room air and ii) rats exposed to CNH (12 % O_2, 3 weeks). Echocardiographic examination of the left ventricle (LV) was performed 3 days before surgery and 7, 14 and 28 days post-MI. MI resulted in a gradual increase in LV end-diastolic diameter (LVD_d) compared to sham-operated animals. Fractional shortening (FS) decreased from 42.8 % before MI to 15.1 % on day 28 post-MI. CNH significantly attenuated ventricular dilatation without affecting scar area and FS. Our data suggest that prolonged exposure to CNH has certain potential to attenuate the progression of unfavorable changes in ventricular geometry induced by MI in rats.

Key words

Chronic hypoxia • Heart failure • Myocardial infarction

Corresponding author

J. Hrdlička, Department of Developmental Cardiology, Institute of Physiology, Czech Academy of Sciences, Vídeňská 1083, 142 20 Prague 4, Czech Republic. E-mail: jaroslav.hrdlicka@biomed.cas.cz

It has been well established that adaptation to chronic hypoxia confers long-lasting cardioprotection against various manifestations of injury caused by acute ischemia/reperfusion (I/R) insult. Increasing evidence has indicated that ischemia-resistant cardiac phenotype can be induced by both continuous hypoxia (Neckář *et al.* 2013, Chytilová *et al.* 2015) and certain regimens of intermittent hypoxia (reviewed in Kolář and Ošťádal 2004). However, much less attention has been paid to potential beneficial effects of chronic hypoxia in secondary prevention of chronic ischemic heart disease and post-infarction heart failure (HF). Del Pilar *et al.* (2006) demonstrated that intermittent hypobaric hypoxia (IHH) improved myocardial perfusion in patients with severe coronary heart disease. Exposure to repeated brief cycles of hypoxia and normoxia for 4 weeks improved cardiac contractile function in a transgenic mouse model of HF (Naghshin *et al.* 2012). Moreover, therapeutic action of IHH on post-infarcted rat hearts has been reported that manifested as a limitation of scar size and attenuation of both progressive myocardial remodeling and contractile dysfunction. These effects can be related to the increased angiogenesis and reduced apoptotic response in the border zone of infarction (Xu *et al.* 2011). It is unknown whether similar beneficial effects can be afforded by continuous hypoxia. Therefore, the aim of this study was to find out whether prolonged continuous exposure of rats to hypoxia ameliorates progression of post-infarction HF.

Adult male Wistar rats (10-week-old, initial

Fax +420 241 062 164, e-mail: physres@biomed.cas.cz, www.biomed.cas.cz/physiolres

body weight 340-390 g, Anlab, Czech Republic) were housed in a controlled environment (23 °C; 12:12-h light-dark cycle) and given water and a standard chow diet *ad libitum*. Animals were randomly assigned into 3 groups: i) sham-operated (Sham), ii) rats with myocardial infarction (MI) and iii) rats with MI exposed to continuous normobaric hypoxia (CNH) (MI/CNH). The study was conducted in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Academy of Science, National Academy Press, Washington D.C.). The experimental protocols were approved by the Animal Care and Use Committee of the Institute of Physiology of the Czech Academy of Sciences.

Acute I/R insult was performed in anesthetized (sodium pentobarbital, 60 mg kg^{-1} i.p.) open-chest rats ventilated with room air essentially as described previously (Neckář *et al.* 2002), except for coronary artery occlusion prolonged to 60 min. Sham surgery was performed identically without occlusion. Then all spontaneously breathing animals recovering from anesthesia were housed in separate cages and received analgesis (Ibuprofen, 6 mg/day p.o.) for another two days.

Since day 7 post-MI, the rats assigned to CNH were housed for 3 weeks in a hypoxic normobaric chamber (12 % O_2) equipped with hypoxic generators (Everest Summit, Hypoxico Inc., NY, USA). A single 30-min reoxygenation period occurred at day 14 for echocardiographic examination.

Echocardiography was performed 3 days before MI and 7, 14 and 28 days post-MI using GE Vingmed System Five (GE Vingmed Ultrasound, Horten, Norway) and FPA 10 MHz probe (GE Vingmed Ultrasound, Horten, Norway). Animals were anesthetized with 2 % isoflurane (Forane; Abbott Laboratories, Queenborough, UK) mixed with room air. Echocardiographic data were recorded and analysed in short and long axis of the heart and in 2D-mode and M-mode. Directly measured LV end-diastolic and end-systolic parameters included cavity diameter (LVD_d and LVD_s), anterior wall thickness (AWT_d and AWT_s) and posterior wall thickness (PWT_d and PWT_s). Based on the LV dimension, fractional shortening (FS) was derived as follows: $FS\ (\%) = [100\times(LVD_d\text{-}LVD_s)/(LVD_d)]$. Values of heart rate (HR) were averaged from at least 4 heart cycles.

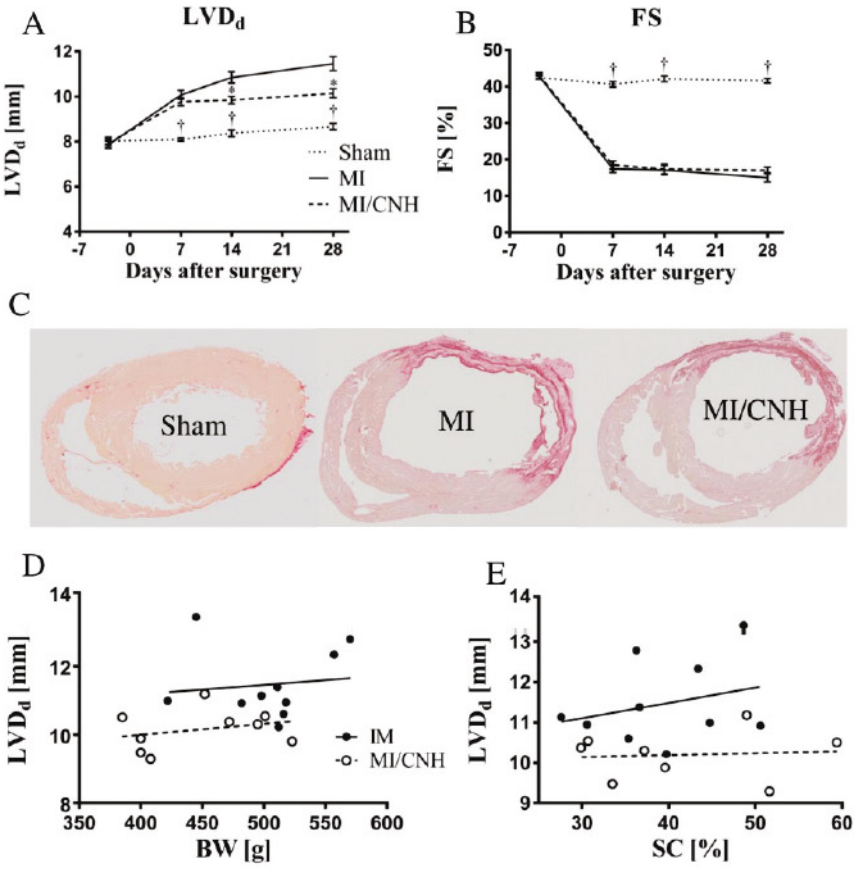

Fig. 1. Left ventricular (LV) echocardiographic parameters and histology of hearts from sham-operated rats (Sham), rats with myocardial infarction (MI) and rats with MI exposed to continuous normobaric hypoxia (MI/CNH). (**A**) LV end-diastolic diameter (LVD_d), (**B**) Fractional shortening (FS), (**C**) Examples of transverse sections of hearts stained with Picrosirius Red for scar delineation at day 28 after surgery, (**D**) Relationship between body weight (BW) and LVD_d at day 28 post-MI, (**E**) Relationship between scar circumference (SC) and LVD_d at day 28 post-MI. Values are means ± SEM from 9-11 hearts in each group. * $P<0.05$ MI/CNH vs. MI, † $P<0.05$ Sham vs. IM and IM/CNH.

After the last echocardiographic measurement, 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, embedded in paraffin, sectioned (9-µm slices) and stained with Picrosirius Red (Sigma-Aldrich, St. Louis, Missouri, USA). Slices were recorded with Olympus VS 110-S1 microscope (lens magnification 20×; Olympus, Hamburg, Germany) traced with computerized planimetry (OlyVIA 2.4, Olympus; NIS Elements 4.11, Laboratory Imaging, Prague, Czech Republic) and the relative scar area (scar area/LV area) and scar circumference (scar midwall length/LV midwall circumference) were determined (Pfeffer *et al.* 1979) and expressed in percentage.

Data are presented as means ± SEM Statistical evaluations were done using one-way ANOVA with Newman-Keuls post-hoc test using GraphPad Prism 6 (GraphPad Software, San Diego, CA, USA). Differences were considered significant when $P<0.05$.

I/R insult led to 56 % mortality within 48 h post-MI compared to 100 % survival in sham-operated group. Sustained ventricular fibrillation was the main cause of death. Mortality was comparable to earlier reports using similar models of large MI (Pfeffer *et al.* 1985, Bech *et al.* 1990) and did not change since day 2 post-MI. Contraction of LV anterior wall ceased after MI, AWT_d and AWT_s decreased by 17.7 % and 43.1 %, respectively, compared to Sham group at day 7 and did not change significantly till day 28; the exposure to CNH had no effect. No differences in PWT were observed among the groups, except for the 16.1 % increase in PWT_d in MI/CNH compared to Sham group (Table 1). As shown in Fig. 1A, LVD_d progressively increased after MI and CNH significantly attenuated this effect: between days 7 and 28, LVD_d grew by 13.7 % and 3.9 % in MI and MI/CNH groups, respectively. Corresponding changes occurred in LVD_s (Table 1). FS dropped markedly after MI but no beneficial effect of CNH was observed (Fig. 1B). Histological analysis did not reveal any effect of CNH on scar area (21.0±1.1 % and 19.8±2.0 % in MI and MI/CNH, respectively) and scar circumference (39.4±2.4 % and 39.4±3.9 % in MI and MI/CNH, respectively) (Fig. 1C).

Table 1. Body weight (BW) and left ventricular (LV) echocardiographic parameters of hearts from sham-operated rats (Sham), rats with myocardial infarction (MI) and rats with MI exposed to continuous normobaric hypoxia (MI/CNH).

Days after surgery	Group	BW (g)	HR (bpm)	LVD_s (mm)	AWT_d (mm)	AWT_s (mm)	PWT_d (mm)	PWT_s (mm)
-3	Sham	365±19	377±8	4.65±0.09	2.14±0.09	3.28±0.08	1.82±0.03	2.86±0.05
	MI	379±22	382±14	4.52±0.13	2.13±0.06	3.30±0.08	1.95±0.06	3.01±0.08
	MI/CNH	341±25	375±8	4.53±0.12	2.04±0.07	3.26±0.10	1.86±0.07	2.67±0.09
7	Sham	425±12	387±7	4.83±0.06†	2.26±0.06†	3.34±0.09†	1.92±0.04	2.93±0.06
	MI	409±17	378±8	8.34±0.25	1.86±0.07	1.90±0.07	1.95±0.09	2.92±0.09
	MI/CNH	382±20	362±6	7.97±0.18	1.91±0.11	1.96±0.12	2.11±0.06	2.96±0.12
14	Sham	465±13‡	377±7	4.87±0.14†	2.33±0.07†	3.56±0.08†	2.04±0.09	3.15±0.05
	MI	446±16	390±8	9.02±0.34	1.81±0.06	1.77±0.05	2.00±0.04	3.05±0.07
	MI/CNH	400±22	365±9	8.13±0.19*	1.84±0.06	1.84±0.06	2.13±0.08	3.12±0.14
28	Sham	527±12‡	367±5	5.09±0.07†	2.25±0.07†	3.54±0.09†	1.99±0.06‡	3.17±0.07
	MI	503±14	376±7	9.77±0.39	1.81±0.03	1.83±0.04	2.12±0.06	3.11±0.06
	MI/CNH	448±17*	341±15*	8.43±0.22*	1.84±0.06	1.89±0.07	2.31±0.08	3.24±0.08

Values are means ± SEM from 9-11 rats in each group. HR, heart rate; LVD_s, end-systolic LV diameter; AWT_d, end-diastolic anterior wall thickness; AWT_s, end-systolic anterior wall thickness; PWT_d, end-diastolic posterior wall thickness; PWT_s, end-systolic posterior wall thickness. * $P<0.05$ MI/CNH vs. MI, † $P<0.05$ Sham vs. MI and MI/CNH, ‡ $P<0.05$ Sham vs. MI/CNH.

As adaptation to CNH resulted in lower body weight compared to MI group (Table 1), it can be argued that body growth retardation was responsible for the attenuation of MI-induced LV dilatation (Litwin *et al.* 1994). However, the relationship between body weight and LVD_d (Fig. 1D) clearly shows that it cannot fully explain the beneficial effect of CNH. Similar attenuation of LV dilatation has been observed in infarcted rats

exposed to chronic IHH which did not affect body weight (Xu *et al.* 2011). It also should be noted that the CNH-induced LVD_d lowering compared to MI group was independent of the proportion of scar tissue (Fig. 1E). In contrast to the study of Xu *et al.* (2011), we did not observe any improvement of FS in infarcted rats adapted to CNH. The reason for this discordance is unknown but differences between models and protocols of IHH and CNH can play a role. In addition, it cannot be excluded that the large extent of MI injury in our study already exceeded the limits of potential effective amelioration of systolic function by chronic hypoxia.

In conclusion, the prolonged exposure of rats to CNH has certain therapeutic potential against the unfavorable changes in ventricular geometry induced by severe MI. Further studies are needed to optimize CNH protocol in order to alleviate the progression of MI-induced heart failure.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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Research Article

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

Jan Neckář[1,2,3], Md. Abdul Hye Khan[1], Garrett J. Gross[1], Michaela Cyprová[2], Jaroslav Hrdlička[2], Alena Kvasilová[4], John R. Falck[5], William B. Campbell[1], Lenka Sedláková[2], Šárka Škutová[2], Veronika Olejníčková[2,4], Martina Gregorovičová[2], David Sedmera[2,4], František Kolář[2] and John D. Imig[1]

[1]Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI, U.S.A.; [2]Department of Developmental Cardiology, Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic; [3]Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; [4]Institute of Anatomy, First Faculty of Medicine, Charles University, Prague, Czech Republic; [5]Department of Biochemistry, University of Texas Southwestern Medical Center, Dallas, TX, U.S.A.

Correspondence: Jan Neckář (jan.neckar@fgu.cas.cz)

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 2 weeks of treatment, rats were subjected to 30-min left coronary artery occlusion or sham operation. Systolic blood pressure (SBP) and echocardiography (ECHO) 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. Fractional shortening (FS) was decreased to 18.4 $\pm$ 1.0% in vehicle-treated MI rats compared with corresponding sham (30.6 $\pm$ 1.0%) 7 weeks following MI induction. In infarcted SHR hearts, EET-B treatment improved FS (23.7 $\pm$ 0.7%), markedly increased heme oxygenase-1 (HO-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.

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 into biologically inactive dihydroxyeicosatrienoic acids

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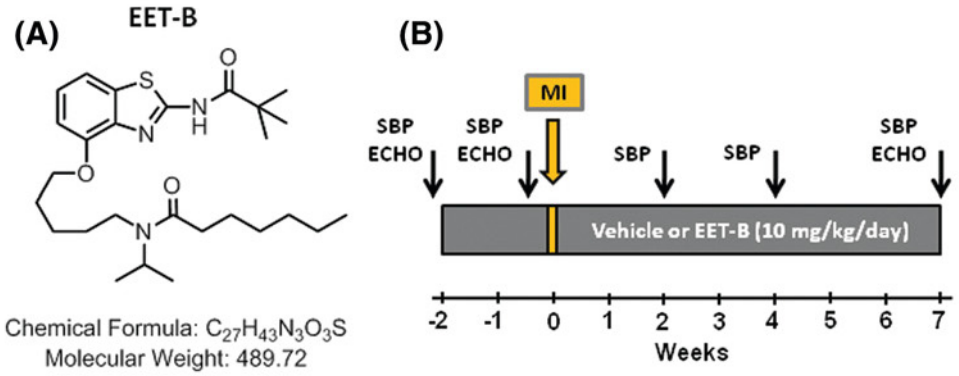

Figure 1. Structure of EET-B and the design of the study

Structure of EET-B (**A**). The design of the experiments analyzing the cardioprotective 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 'Methods' section for the detailed description.

(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, cardio-protective effects of a novel, stable and orally active agonistic 14,15-epoxyeicosatrienoic acid (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 h [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 (SS) 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 SHR were purchased from Charles River Laboratories (Wilmington, MA, U.S.A.). Animals were housed in the Biomedical Resource Center at Medical College of Wisconsin with a 12/12-h 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 a.m.) for 3 days before every intervention.

Echocardiographic assessment of cardiac function

Echocardiography (ECHO) 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, U.K.) mixed with room air. Prior to starting the treatment protocol, ECHO measurements were performed at baseline and then 4 days before and 7 weeks after MI or sham operation in anesthetized rats (Figure 1B). Diastolic and systolic dimensions of LV were measured during ECHO evaluation including anterior and posterior wall thickness (diastolic anterior wall thickness (AWTd), diastolic posterior wall thickness (PWTd), systolic anterior wall thickness (AWTs), systolic posterior wall thickness (PWTs)) and LV cavity diameter (diastolic LV diameter ((LVDd), systolic LV diameter (LVDs)). From these dimensions, the following functional ECHO parameters were derived: fractional shortening (FS) using the formula (LVDd − LVDs)/LVDd*100, systolic thickening of anterior wall (ΔAWT) from the equation (AWTs − AWTd)/AWTd*100, and relative wall thickness (RWT) from the equation (AWTd + PWTd)/LVDd*100.

Myocardial ischemia

After the second ECHO 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 2 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 (ventricular 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 (ventricular 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 ECHO measurements, rats were killed 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-μm slices for use in histology protocols. Formalin-fixed and paraffin-embedded tissue slices were de-paraffinized, re-hydrated and stained with Hematoxylin–Eosin or Picro-Sirius 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, U.K.) and anti-CD68 (1:50; Serotec, Raleigh, NC, U.S.A.) to detemine macrophage/monocyte infiltration. Horseradish peroxidase–conjugated goat anti-rabbit secondary antibody (1:200; Jackson ImmunoResearch Laboratories, West Grove, PA, U.S.A.) was used for development with avidin-biotinylated HRP complex (Vectastain ABC Elite kit, Vector Laboratories, Burlingame, CA, U.S.A.) followed by counterstaining with Hematoxylin. Stained sections were visualized by light microscopy at 400× magnification and digital images of the stained sections were taken for analysis using Nikon NIS Elements Software (Nikon Instruments Inc., Melville, NY, U.S.A.). Collagen content and HO-1 expression were determined by quantitation of Picro-Sirius 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 ± 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 PVCs between the groups were compared by Mann–Whitney test. The incidence of VT and VF 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).

Clinical Science (2019) **133** 939–951
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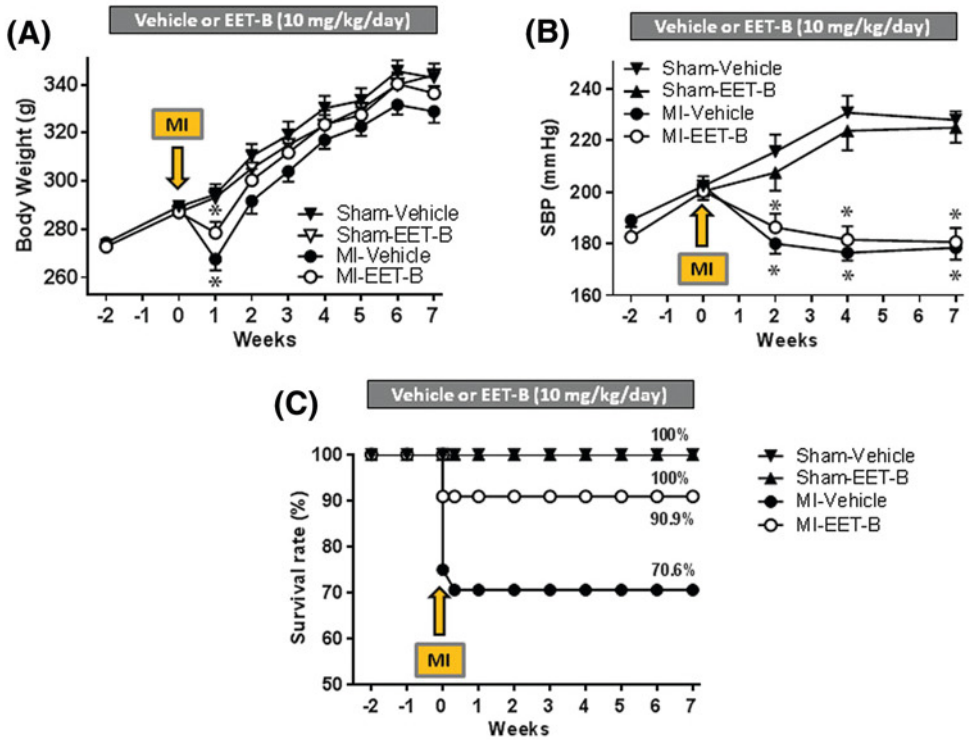

Figure 2. Body weight, systolic blood pressure and survival rate

Body weight (**A**), systolic blood pressure (SBP) (**B**) and survival rate (**C**) in vehicle- and EET-B-treated SHR subjected to MI and sham-operated groups. Rats were treated by vehicle or EET-B (10 mg/kg/day) from 2 weeks before till 7 weeks after MI. Values are means ± SEM from 6–12 rats in each group. *$P<0.05$ vs. corresponding sham-operated group.

Table 1 PVCs occurring as singles, salvos and VT, incidence of VT and VF and duration of tachyarrhythmias (VT+VF) during 30-min of coronary artery occlusion in vehicle- and EET-B-treated hearts of SHR

Group	Number of arrhythmias					Incidence (%)		Duration of tachyarrhythmias (s)
	n	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)

Values are median (range). Abbreviation: *n*, number of animals.

In both MI groups, a significant reduction in BW was evident 1 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 7 weeks following MI due to heart failure in both vehicle- and EET-B-treated groups compared with corresponding sham-operated rats.

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

VFs 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 h 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 with vehicle-treated SHR but the differences did not reach statistical significance (Table 1).

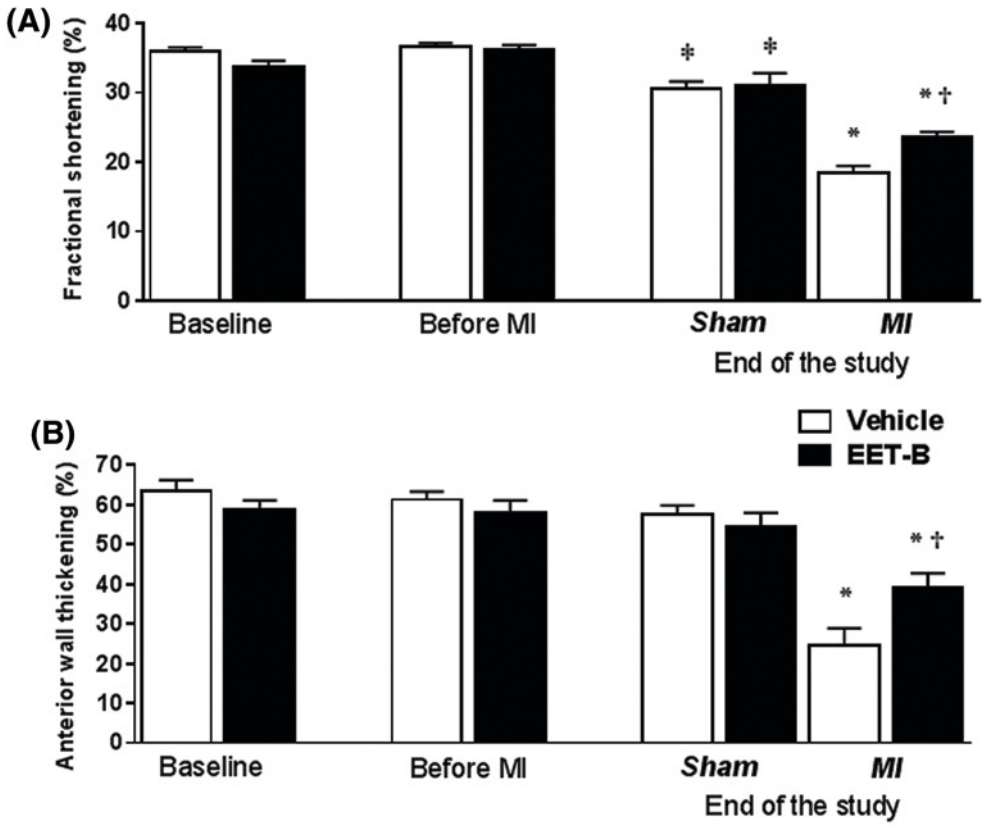

Figure 3. Echocardiographic parameters

ECHO assessment of left ventricular fractional shortening (**A**) and the anterior wall systolic thickening (**B**) in vehicle- and EET-B-treated SHR subjected to MI and sham-operated groups at the start of study (Baseline), after 10 days of treatment (Before MI) and 7 weeks after MI (End of the study). Rats were treated by vehicle or EET-B from 2 weeks before until 7 weeks after MI. Values are means ± 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.

Table 2 Echocardiographic parameters of LV in vehicle- and EET-B-treated SHR subjected to 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).

	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

Values are means ± SEM from 6–10 rats in each group. Abbreviations: bpm, beats per minute; HR, heart rate.
*$P<0.05$ vs. corresponding sham group.
†$P<0.05$ MI-EET-B vs. MI-vehicle group.
‡$P<0.05$ vs. corresponding Before MI group.

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 ECHO were not altered by vehicle or EET-B treatment prior to MI. A significant increase in LVDs and LVDd were found in sham vehicle- and

Clinical Science (2019) **133** 939–951
https://doi.org/10.1042/CS20180728

Table 3 Relative weights of lung and heart 7 weeks after MI in vehicle- and EET-B-treated SHR subjected to MI and sham operation

	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.

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 with values prior to surgery (Figure 3A). In the SHR group subjected to MI and vehicle-treated, both FS and ΔAWT were markedly decreased to 18.4 ± 1.0 and 24.5 ± 4.4%, respectively, compared with age-matched sham-operated controls that averaged 30.6 ± 1.0 and 57.5 ± 2.6%, respectively. EET-B treatment significantly attenuated MI-induced reductions in FS and ΔAWT to 23.7 ± 0.7 and 39.1 ± 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 with 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 ± 1.5 and 2.18 ± 0.11%, respectively (Figure 4). EET-B treatment significantly reduced myocardial fibrosis and CD68 positive area to 37.2 ± 1.9 and 1.77 ± 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 ± 0.08 and 0.25 ± 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 ± 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 MI

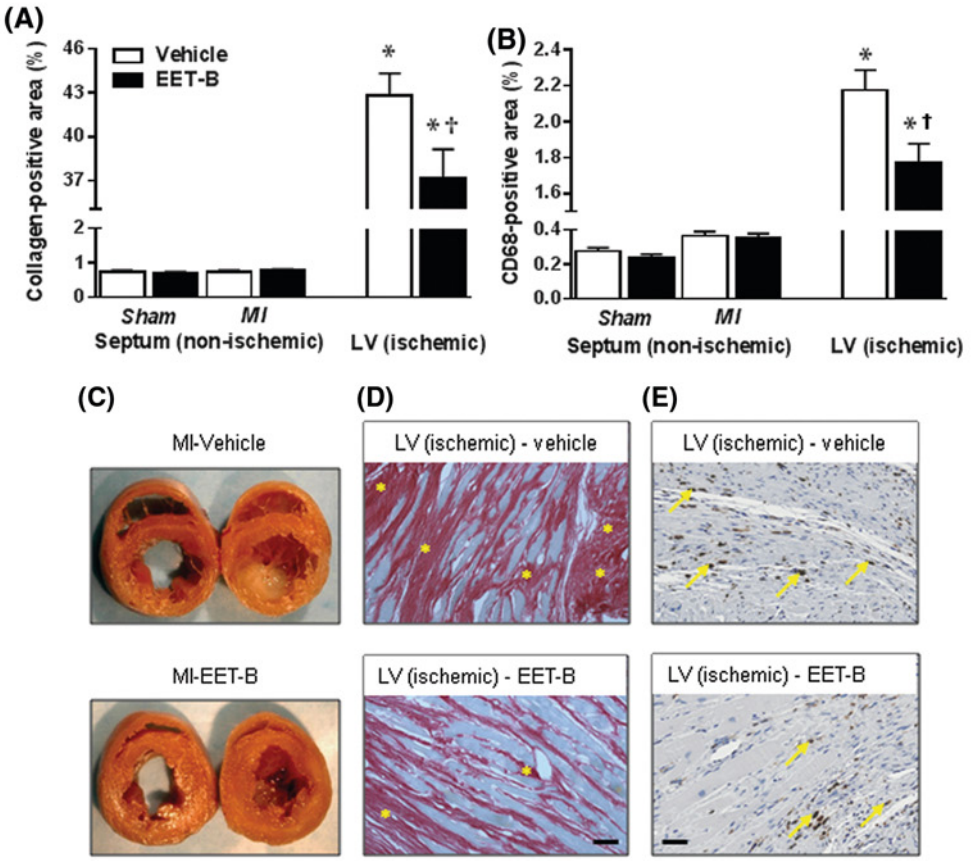

Figure 4. Myocardial fibrosis and inflammation

Quantitation of myocardial collagen-positive area (**A**) and CD68-positive area (**B**) assessed in non-ischemic septum, and ischemic area of LV of vehicle- and EET-B-treated SHR subjected to 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 2 weeks before till 7 weeks after MI. Scale bars represent 50 μm. Values are means ± SEM from 6–10 rats in each group. *$P<0.05$ vs. sham-vehicle group. †$P<0.05$ vs. MI-vehicle group.

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 with 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 2-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-threating ischemic arrhythmias in transgenic *Ren-2* rats with angiotensin II-dependent hypertension [19] which is consistent with the anti-arrhythmic trend 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. The hypertension severity before MI and the reduction in 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 SS hypertensive rats [15]. The ability for EET-B to exert a blood pressure-lowering action in Dahl SS rats and in SHR 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

Clinical Science (2019) **133** 939–951
https://doi.org/10.1042/CS20180728

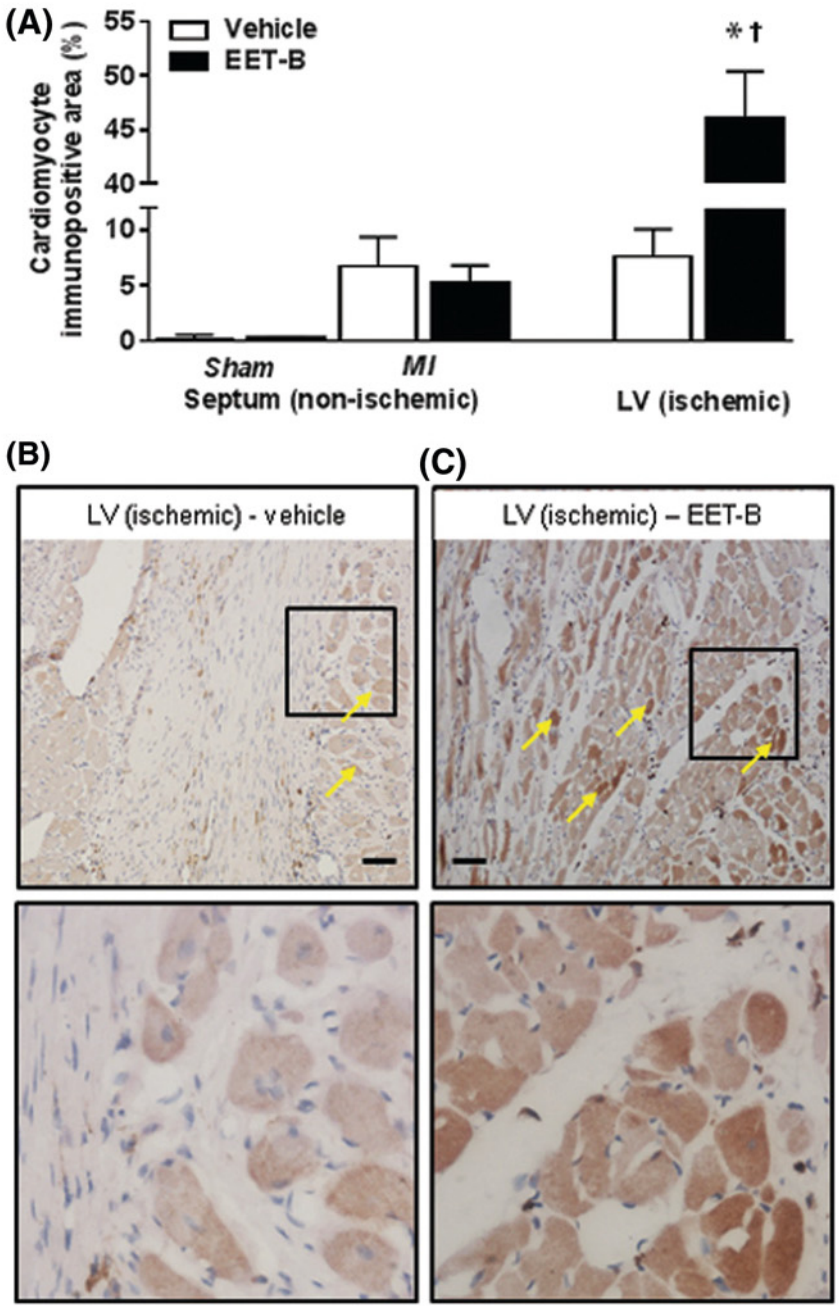

Figure 5. Heme oxygenase-1

Quantitation of HO-1 immunopositivity in viable cardiomyocytes assessed in non-ischemic septum, and ischemic area of LV of vehicle- and EET-B-treated SHR subjected to 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 2 weeks before till 7 weeks after MI. Scale bars represent 75 μm. Values are means ± SEM from 6–10 rats in each group. *$P<0.05$ vs. sham-vehicle group. †$P<0.05$ vs. MI-vehicle group.

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 cardio-protective ability of EET-B. However, it should be noted that in humans after MI, the published findings are not completely clear with respect to EET cardio-protective actions on the progression of heart dysfunction. 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 were 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 2 weeks before MI and continued till 7 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 in life-threating arrhythmias [19]. 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]. These findings support previous reports indicating that the protective action of EET analogs against various cardiovascular diseases is independent of their anti-hypertensive actions.

The effect of EET-based therapy on the progression of CHF-associated etiologies other than ischemic heart disease is rather inconsistent. Indeed, it has been reported that sEH inhibitors can reduce [11,42] or unchange [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 ΔAWT compared with vehicle-treated SHR 7 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 that was reduced by EET-B treatment. Taken together, in post-MI SHR, EET-B-mediated improvement of LV systolic function was 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. It 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

Clinical Science (2019) **133** 939–951
https://doi.org/10.1042/CS20180728

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 attenuated the progression of post-MI LV dysfunction [60] and reduced 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 suggest that HO-1 is a contributing factor for EET-B mediated reduction in 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 a tendency to lower incidence and severity of ischemic ventricular arrhythmias, reduced lung edema, LV systolic dysfunction, myocardial fibrosis and inflammation. Our data also suggest that HO-1 signaling can mediate EET-B cardio-protective actions in SHR with MI-induced CHF.

Clinical 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.

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Author Contribution

J.N. primarily conceived and designed the study, performed experiments, analyzed and interpreted the data and wrote the manuscript. J.D.I., G.J.G. and M.A.H.K. designed the study and wrote the manuscript. J.D.I., J.R.F. and W.B.C. designed and synthesized EET-B. M.A.H.K., M.C., J.H., A.K., L.S., Š.Š., V.O., M.G., D.S. and F.K. 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 Authors declare that there are no competing interests associated with the manuscript.

Abbreviations

AWTd, diastolic anterior wall thickness; AWTs, systolic anterior wall thickness; ΔAWT, systolic thickening of anterior wall; BW, body weight; CHF, congestive heart failure; DHET, dihydroxyeicosatrienoic acid; 14,15-DHET, 14,15-dihydroxyeicosatrienoic acid; ECHO, echocardiography; EET, epoxyeicosatrienoic acid; 14,15-EET, 14,15-epoxyeicosatrienoic acid; FS, fractional shortening; HO-1, heme oxygenase-1; LV, left ventricle; LVDd, diastolic LV diameter; LVDs, systolic LV diameter; MI, myocardial infarction; PVC, premature ventricle complex; PWTd, diastolic posterior wall thickness; SBP, systolic blood pressure; sEH, soluble epoxide hydrolase; SHR, spontaneously hypertensive rat; SS, salt-sensitive; VF, ventricular fibrillation; VFs, sustained VF; VT, ventricular tachycardia.

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Clinical Science (2019) **133** 939–951
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Epoxyeicosatrienoic Acid-Based Therapy Attenuates the Progression of Postischemic Heart Failure in Normotensive Sprague-Dawley but Not in Hypertensive *Ren-2* Transgenic Rats

Jaroslav Hrdlička[1,2], Jan Neckář[1,3], František Papoušek[1], Zuzana Husková[3], Soňa Kikerlová[3], Zdenka Vaňourková[3], Zdenka Vernerová[3], Firat Akat[1,4], Jana Vašinová[1], Bruce D. Hammock[5], Sung Hee Hwang[5], John D. Imig[6], John R. Falck[7], Luděk Červenka[3] and František Kolář[1]*

[1] Institute of Physiology of the Czech Academy of Sciences, Prague, Czechia, [2] Department of Physiology, Faculty of Science, Charles University, Prague, Czechia, [3] Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Prague, Czechia, [4] Department of Physiology, Faculty of Medicine, Ankara University, Ankara, Turkey, [5] Department of Entomology and Nematology, UC Davis Comprehensive Cancer Center, University of California, Davis, Davis, CA, United States, [6] Department of Pharmacology and Toxicology, Medical College of Wisconsin, Milwaukee, WI, United States, [7] Department of Biochemistry, University of Texas Southwestern, Dallas, TX, United States

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Edited by:
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***Correspondence:**
Jan Neckář
jan.neckar@fgu.cas.cz

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Epoxyeicosatrienoic acids (EETs) and their analogs have been identified as potent antihypertensive compounds with cardio- and renoprotective actions. Here, we examined the effect of EET-A, an orally active EET analog, and *c*-AUCB, an inhibitor of the EETs degrading enzyme soluble epoxide hydrolase, on the progression of post-myocardial infarction (MI) heart failure (HF) in normotensive Hannover Sprague-Dawley (HanSD) and in heterozygous *Ren-2* transgenic rats (TGR) with angiotensin II-dependent hypertension. Adult male rats (12 weeks old) were subjected to 60-min left anterior descending (LAD) coronary artery occlusion or sham (non-MI) operation. Animals were treated with EET-A and *c*-AUCB (10 and 1 mg/kg/day, respectively) in drinking water, given alone or combined for 5 weeks starting 24 h after MI induction. Left ventricle (LV) function and geometry were assessed by echocardiography before MI and during the progression of HF. At the end of the study, LV function was determined by catheterization and tissue samples were collected. Ischemic mortality due to the incidence of sustained ventricular fibrillation was significantly higher in TGR than in HanSD rats (35.4 and 17.7%, respectively). MI-induced HF markedly increased LV end-diastolic pressure (P_{ed}) and reduced fractional shortening (FS) and the peak rate of pressure development [$+(dP/dt)_{max}$] in untreated HanSD compared to sham (non-MI) group [P_{ed}: 30.5 ± 3.3 vs. 9.7 ± 1.3 mmHg; FS: 11.1 ± 1.0 vs. 40.8 ± 0.5%; $+(dP/dt)_{max}$: 3890 ± 291 vs. 5947 ± 309 mmHg/s]. EET-A and *c*-AUCB, given alone, tended to improve LV function parameters in HanSD rats. Their combination amplified the cardioprotective effect of single therapy and reached significant differences compared to untreated HanSD controls [P_{ed}: 19.4 ± 2.2 mmHg; FS: 14.9 ± 1.0%; $+(dP/dt)_{max}$: 5278 ± 255 mmHg/s].

In TGR, MI resulted in the impairment of LV function like HanSD rats. All treatments reduced the increased level of albuminuria in TGR compared to untreated MI group, but neither single nor combined EET-based therapy improved LV function. Our results indicate that EET-based therapy attenuates the progression of post-MI HF in HanSD, but not in TGR, even though they exhibited renoprotective action in TGR hypertensive rats.

Keywords: epoxyeicosatrienoic acid, soluble epoxide hydrolase, chronic heart failure, hypertension, myocardial infarction, echocardiography

INTRODUCTION

Current experimental and clinical research into pathophysiological mechanisms of cardiovascular diseases provided series of evidences suggesting an increased activity of soluble epoxide hydrolase (sEH) in several heart and kidney disorders (Imig, 2012; Oni-Orisan et al., 2014; Campbell et al., 2017). sEH is an enzyme responsible for rapid conversion of cytochrome P450 arachidonic acid epoxygenase metabolites, the epoxyeicosatrienoic acids (EETs), to inactive or less active dihydroxyeicosatrienoic acids (DHETs). It has been shown that EETs have beneficial action to combat many cardiovascular diseases and their progression including hypertension, ischemic heart diseases, chronic heart failure (CHF), diabetes mellitus, chronic kidney diseases etc. (reviewed in Qiu et al., 2011; Jamieson et al., 2017; Imig, 2018). Therefore, sEH inhibitors represent a potential class of drugs for treating various cardiovascular diseases.

Despite major improvements in the therapy of cardiac disorders, the prevalence of CHF is rising (Braunwald, 2013). In most instances, CHF is the irreversible and final consequence of heart injury associated with high morbidity and mortality. The progression of left ventricle (LV) dysfunction following acute myocardial infarction (MI) is a predominant cause of CHF (Roger, 2013). Regarding the therapeutic effects of EETs on post-MI remodeling, it has been shown that chronic treatment of normotensive rats or mice with sEH inhibitors reduce the progression of LV systolic dysfunction (Li et al., 2009; Merabet et al., 2012; Kompa et al., 2013; Sirish et al., 2013).

It is well known that specific metabolic pathways, including sEH, quickly terminate the biological activity of EETs (Spector and Norris, 2007). To circumvent this limitation of endogenous EETs, several synthetic and more stable EET analogs with markedly longer half-life and promising cardioprotective actions have been developed (Campbell et al., 2017). Previously, it has been shown that NUDSA, the first generation EET analog, increased LV function and decreased cardiac fibrosis in mice after MI (Cao et al., 2015).

Altogether, the EET therapy based on EET analogs or sEH inhibitors can limit a harmful myocardial remodeling associated with the progression of post-MI CHF, however, their combined action was never analyzed. Therefore, here we examined the effect of EET-A (a third generation of EET analog with better water solubility and improved oral bioavailability) and *c*-AUCB (sEH inhibitor), given alone or combined, on the progression of post-MI CHF in normotensive Hannover Sprague-Dawley (HanSD) and in heterozygous *Ren-2* transgenic rats (TGR) with angiotensin II (Ang II)-dependent form of hypertension. Previously, it has been shown that *c*-AUCB reduced mortality in TGR subjected to aorto-caval fistula, a well-defined model of CHF due to volume overload (Červenka et al., 2015a). Moreover, the same EET-based therapy (EET-A and *c*-AUCB) administered before MI effectively reduced high blood pressure and decreased the incidence of life-threatening ventricular fibrillation in hypertensive TGR (Červenka et al., 2018).

MATERIALS AND METHODS

Animals and Experimental Protocol

HanSD rats and TGR were bred at the Center of Experimental Medicine of the Institute for Clinical and Experimental Medicine in Prague and housed in a controlled environment (23°C, 12 h light-dark cycle; light from 6:00 AM) with free access to water and standard chow diet. The study was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Academy of Science, National Academy Press, Washington, DC. The experimental protocols were approved by the Animal Care and Use Committee of the Institute of Physiology of the Czech Academy of Sciences.

Adult male HanSD rats and TGR (12 weeks old; $n = 76$ and 90, respectively) were subjected to 60-min regional ischemia or sham operation as described earlier (Hrdlička et al., 2016). Briefly, animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p., Sigma-Aldrich, United States). Intubated rats were ventilated (Ugo Basile, Italy) with room air at 68 strokes/min (tidal volume of 1.2 ml/100 g body weight) and their rectal temperature was maintained between 36.5 and 37.5°C with a heating pad. A left thoracotomy was performed and the pericardium was removed to reveal the location of the left anterior descending (LAD) coronary artery. Then a silk ligature 6/0 (Chirmax, Czech Republic) was placed around the LAD coronary artery about 1–2 mm distal to the origin and an occlusive snare was placed around it. The ends of the suture were threaded through a polyethylene tube. After the surgical preparation, the rats were allowed to stabilize for 10 min before the ischemic intervention. Myocardial ischemia was induced by tightening the ligature around the coronary artery. Sham (non-MI) surgery was performed identically without occlusion. Characteristic changes in myocardial color and the incidence of ischemic arrhythmias verified the complete coronary artery occlusion. At the start of reperfusion, the snare was released, 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 (Ibuprofen, 6 mg/day p.o.) for 3 consecutive days.

Twenty-four hours after surgery, rats of both strains were randomly assigned based on their treatment to the following groups: (i) untreated Sham-operated (non-MI), (ii) post-MI untreated controls, and rats treated by (iii) EET-A (10 mg/kg/day, p.o.), (iv) *c*-AUCB (1 mg/kg/day, p.o.), and (v) a combination of EET-A and *c*-AUCB (10 mg/kg/day and 1 mg/kg/day, respectively, p.o.).

Echocardiography

LV geometry and function were assessed by echocardiography 3 days prior to MI and 5 weeks post-MI using GE Vivid 7 Dimension (GE Vingmed Ultrasound, Horten, Norway) with a 12 MHz linear matrix probe M12L (Hrdlička et al., 2016). Animals were anesthetized with 2% isoflurane (Forane, Abbott Laboratories, Queenborough, United Kingdom) mixed with room air, placed on a heating pad and their rectal temperature was maintained between 36.5 and 37.5°C. Basic 2-D and M-mode in both long and short axis and 4-D mitral flow and pulmonary artery (PA) pulse Doppler measurements were recorded. Heart rate (HR) and following parameters of LV geometry were assessed: end-diastolic and systolic LV cavity diameter (LVDd, LVDs), LV cavity length (LVLd, LVLs), LV cavity area in short axis (LVAd, LVAs), anterior wall thickness (AWTd, AWTs), and posterior wall thickness (PWTd, PWTs). Fractional shortening (FS) and relative wall thickness (RWT) were derived as follows: FS = 100*[(LVDd-LVDs)/LVDd]; RWT = 100*[(AWTd+PWTd)/LVDd].

Heart Catheterization

At the end of the study, rats anesthetized with 2% isoflurane were subjected to LV catheterization through the right carotid artery using the SPR-407 microtip pressure catheter as described previously (Lee et al., 2008) and data were acquired using MPVS 300 (Millar, Houston, TX, United States), PowerLab 8/30 (ADInstruments, Oxford, United Kingdom). End-diastolic, systolic, and developed pressure and peak rate of pressure development and decline (+(dP/dt)max, -(dP/dt)max, respectively) were assessed from 5 consecutive pressure cycles using LabChart Pro (ADInstruments, Oxford, United Kingdom).

Plasma Monocyte Chemoattractant Protein-1 (MCP-1)

After catheterization, blood was collected from the right ventricle, centrifuged and plasma samples were frozen in liquid nitrogen and stored in -80°C. Plasma concentration of MCP-1 was measured by a quantitative sandwich enzyme immunoassay technique, using a commercially available ELISA kit (BMS631INST, Invitrogen by Thermo Fischer Scientific, Austria).

Kidney Injury Markers

Twenty-four hour urine samples were collected at the end of the five-week post-MI follow-up period. Albumin and cystatin C were measured by a quantitative sandwich enzyme immunoassay technique, using a commercially available ELISA kits (ERA3201-1, AssayPro, MO, United States; EK1109, BOSTER Biological Technology Co., Ltd., CA, United States). Urine creatinine was determined using Liquick Cor-Creatinine kit without deproteinization (PZ Cormay S.A., Poland). In alkaline solution, picrate reacts with creatinine to form a yellow-red 2,4,6-trinitrocyclohexadienate. The color intensity measured by a photometer at 500 nm is proportional to the creatinine concentration. Albumin data were normalized to the creatinine data. Sodium and potassium levels in urine samples were measured using flame photometer BWB-XP (BWB Technologies, Great Britain).

Kidneys were immersion-fixed in 10% neutral buffered formalin and paraffin embedded. Tissue sections were cut into 4 μm slices for use in histology protocols. Tissue slices were de-paraffinized, re-hydrated and stained with hematoxylin/eosin and periodic acid-Schiff reaction and examined using Nikon Eclipse Ni-E. Slides were evaluated in a blind fashion. Renal damage was expressed as a total index, the sum of glomerulosclerosis and cortical tubulointerstitial injury (CTI).

Glomerulosclerosis/hyalinosis (GSI) was defined as segmental solidification of glomerular capillary tuft, segmental collapse, obliteration of capillary lumen and accumulation of hyaline. One hundred glomeruli per section were randomly selected and evaluated using semiquantitative scoring method. Degree of sclerosis in each glomerulus was graded subjectively: grade 0, normal; grade 1, sclerotic area up to 25% (minimal); grade 2, sclerotic area 25–50% (moderate); grade 3, sclerotic area 50–75% (moderate-severe); and grade 4, sclerotic area 75–100% (severe). GSI index was calculated using the following formula: GSI = [(1*n1)+(2*n2)+(3*n3)+(4*n4)]/n0+n1+n2+n3+n4, where n is the number of glomeruli in each grade of glomerulosclerosis.

Cortical tubulointerstitial injury (CTI) was defined as inflammatory cell infiltration, tubular dilatation and/or atrophy, or interstitial fibrosis. Lesions were assessed for at least 30 random and non-overlapping fields in a cortex and were graded semiquantitatively using the following scale: 0, no abnormal findings; 1, mild (up to 25% of the cortex); 2, moderate (25–50% of the cortex); 3, severe (more than 50% of cortex). CTI index was calculated using the following formula: CTI = [(1*n1)+(2*2n)+(3*3n)]/30 (fields), where nx is the number of fields in each grade.

Statistical Analysis

Data are expressed as mean ± SEM. Statistical evaluation were done using GraphPad Prism 6 (GraphPad Software, San Diego, CA, United States). The incidence of mortality was evaluated by Fisher's exact test. For multiple comparison of between group differences one-way analysis of variance (ANOVA) and Holm-Sidak's multiple comparison *post hoc* test were used. The values exceeding 95% probability limits ($P < 0.05$) were considered statistically significant.

RESULTS

Weight Parameters, Mortality, and Plasma MCP-1 Level

At the beginning of the study, the experimental groups did not differ in body weight (BW). A slight reduction in BW was observed one week after MI. From week two post-MI, BW gain occurred and by the end of the study BW did not differ among the groups (**Figures 1A,B**).

In HanSD rats, the acute ischemic mortality was 17.7% due to the incidence of sustained ventricular fibrillation (sVF); mortality increased to 25.0% by 24 h after MI (**Figures 1C,D**). Only two additional untreated HanSD rats died during 5 weeks of post-MI period. In TGR, the acute and 24 h mortality were significantly higher (35.4 and 42.7%, respectively) compared to HanSD rats (**Figures 1C,D**). Moreover, three untreated rats and four rats from each therapy group of TGR died during post-MI period; the post-MI mortality did not differ among TGR groups. Overall, 72.1% (49 out of 68) of HanSD rats and 39.0% (32 out of 82) of TGR subjected to MI survived till the end of the study.

As summarized in **Table 1**, in untreated HanSD rats MI led to the significant increase in the relative heart weight (HW/BW) compared to Sham (non-MI) group (3.23 ± 0.14 mg/g vs. 2.71 ± 0.14 mg/g). Sham (non-MI) TGR exhibited higher HW/BW (3.22 ± 0.11 mg/g) than HanSD rats. As compared to HanSD rats, MI had no significant effect on the relative heart weight in TGR (**Table 1**). Neither single nor combined EET-based therapy significantly affected HW/BW in both strains compared to corresponding untreated MI groups.

The progression of post-MI CHF was associated with significant increase in lungs weight by 139 and 123%, respectively, in untreated HanSD rats and TGR compared to corresponding non-MI controls. In HanSD rats (but not in TGR) treated with EET-A alone or combined with *c*-AUCB, relative lung weight increased only by 65 and 63%, respectively (**Table 1**). Nevertheless, the observed decreases did not reach statistical significance compared to untreated HanSD rats.

The concentration of MCP-1, a chemotactic cytokine, was analyzed as a marker of systemic inflammation in plasma. As shown in **Supplementary Figure S1**, neither MI nor EET-based therapy significantly affected MCP-1 levels in both strains at the end of study.

Kidney Injury Markers

Neither MI nor EET-based therapy affected kidney weight, albuminuria, and kidney total injury score in HanSD rats (**Table 1** and **Figure 2**). In untreated TGR, increased levels of kidney injury markers were observed. MI slightly decreased the relative

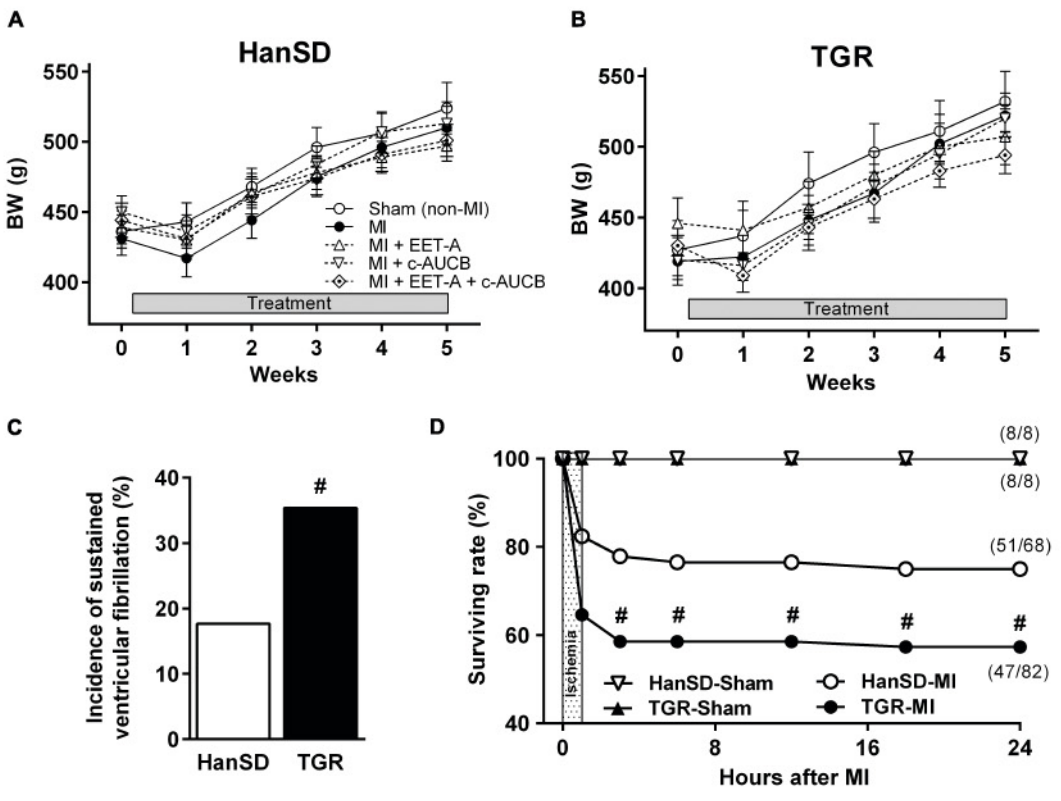

FIGURE 1 | Body weight of Hannover Sprague-Dawley (HanSD; **A**) and *Ren-2* transgenic rats (TGR; **B**) before myocardial infarction (MI) and during 5 weeks of post-MI period and in Sham (non-MI) operated animals. Rats were treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined. The incidence of sustained ventricular fibrillation during ischemia **(C)** and 24 h survival rate **(D)** in HanSD and TGR subjected to MI. Values are means ± SEM. $^{\#}P < 0.05$ vs. HanSD.

kidney weight, albuminuria and kidney total injury score (by 13, 45, and 27%). EET-based therapy had no additional effects on kidney weight. EET-A decreased albuminuria in TGR by 56%, though the effect was not significant. However, *c*-AUCB and the combined treatment reduced albuminuria significantly (by 72 and 87%, respectively). EET-based therapy decreased the total index of kidney injury by 43–57% in TGR (**Figure 2B**) but the differences did not reach statistical significances. Finally, neither MI nor EET-based therapy significantly affected urinary excretion of sodium and potassium as well as cystatin C, a marker of renal tubular dysfunction, at the end of study (**Supplementary Figure S2**).

Heart Geometry and Function Assessed by Echocardiography

At the beginning of the study (before MI), AWTd and PWTd reached 2.10 ± 0.07 mm and 2.09 ± 0.04 mm, respectively, in Sham (non-MI) HanSD rats; LV systolic function, determined as FS, was 42.1 ± 0.9%. In Sham (non-MI) TGR, concentric LV hypertrophy and systolic dysfunction was observed (AWTd: 2.60 ± 0.09 mm; PWTd: 2.61 ± 0.07 mm; FS: 36.9 ± 1.6%). Similar differences in diastolic wall thickness and systolic function between Sham (non-MI) HanSD rats and TGR were also observed at the end of study (**Table 2** and **Figure 3**).

As summarized in **Table 2**, MI without treatment resulted in significant decreases in diastolic and systolic AWT and increased LVD in both strains compared to corresponding Sham (non-MI) groups. In the untreated MI group of HanSD rats, systolic PWT was also significantly decreased. These changes in LV geometry were reflected in significantly decreased RWT in both strains (by 31% in HanSD rats and 48% in TGR; **Table 2**). In HanSD rats (but not in TGR), EET-A or *c*-AUCB, given alone, decreased both diastolic (11.24 ± 0.17 and 11.13 ± 0.16 mm, respectively) and systolic (9.65 ± 0.17 and 9.62 ± 0.20 mm, respectively) LVD compared with untreated MI controls (LVDd: 11.70 ± 0.17; LVDs: 10.37 ± 0.21) but the differences did not reach statistical significances. The combined treatment decreased LVDs significantly (9.50 ± 0.29 mm) compared to untreated MI group. Other parameters of LV geometry were not significantly affected by the treatments in any strain (**Table 2**).

MI markedly decreased LV systolic function expressed as FS in untreated animals of both strains (to 11.1 ± 1.0% and 11.0 ± 0.9%, respectively; **Figure 3**). In HanSD rats, EET-A and *c*-AUCB, given alone, improved FS to 14.1 ± 0.8% and 13.5 ± 0.9%, respectively, but the increase was not statistically significant. The combined administration of EET-A and *c*-AUCB amplified the cardioprotective effect of single therapy and significantly improved FS to 14.9 ± 1.0% compared with untreated HanSD controls. In TGR, neither single nor combined EET-based therapy affected LV systolic function (**Figure 3**).

Progression of post-MI heart failure was associated with changes of mitral flow time parameters. In both untreated HanSD rats and TGR, MI significantly increased the LV filling peak velocity, prolonged the isovolumic contraction time, and shortened the filling time, but had no effect on the ejection time and isovolumic relaxation time (**Table 3**). In HanSD rats, *c*-AUCB alone significantly reduced the prolongation of isovolumic contraction time. Combined EET-based therapy shortened the isovolumic contraction time and also prolonged the filling time compared to MI untreated controls. Neither single nor combined EET-based therapy affected CHF-associated changes in time parameters of mitral flow in TGR.

MI reduced the PA peak and mean velocities, but did not change the PA ejection time and acceleration time in both untreated post-MI groups (**Table 3**). In HanSD rats, the PA mean velocity significantly increased after *c*-AUCB as well as combined treatments. Neither single nor combined EET-based therapy affected CHF-associated changes in PA flow in TGR.

TABLE 1 | Relative weights of lung, heart and right kidney of Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to sham operation (non-MI) or myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined, for 5 weeks since 24 h after MI.

	n	Heart/BW (mg/g)	Lungs/BW (mg/g)	Kidney/BW (mg/g)
HanSD				
Sham (non-MI)	8	2.71 ± 0.14	2.94 ± 0.07	3.20 ± 0.07
MI	11	3.23 ± 0.14†	7.04 ± 0.82†	3.17 ± 0.15
MI + EET-A	14	3.03 ± 0.09	4.84 ± 0.64	3.43 ± 0.06
MI + *c*-AUCB	10	3.10 ± 0.10	6.63 ± 1.28	3.44 ± 0.09
MI + EET-A + *c*-AUCB	14	3.00 ± 0.05	4.78 ± 0.75	3.46 ± 0.04
TGR				
Sham (non-MI)	8	3.22 ± 0.11	3.05 ± 0.13	3.57 ± 0.10
MI	8	3.44 ± 0.11	6.82 ± 0.59†	3.11 ± 0.09†
MI + EET-A	8	3.19 ± 0.08	6.46 ± 0.97	3.19 ± 0.04
MI + *c*-AUCB	10	3.19 ± 0.14	6.93 ± 0.74	3.14 ± 0.09
MI + EET-A + *c*-AUCB	6	3.11 ± 0.11	7.55 ± 1.29	3.07 ± 0.04

BW, body weight; n, number of animals. Values are means ± SEM. †P < 0.05 MI vs. corresponding Sham (non-MI) group.

Heart Function and Blood Pressure Assessed by Catheterization

As demonstrated in **Figure 4**, the progression of post-MI CHF resulted in impaired LV contractile function. In MI untreated HanSD and TGR groups, the peak rate of pressure development $[+(dP/dt)_{max}]$ markedly decreased to 3890 ± 291 and 3485 ± 417 mmHg/s, respectively, compared to corresponding Sham (non-MI) groups (5947 ± 301 and 6910 ± 462 mmHg/s, respectively, **Figure 4A**). In HanSD rats, EET-A or *c*-AUCB, given alone, improved $+(dP/dt)_{max}$ to 4596 ± 297 and 4442 ± 287 mmHg/s, respectively, though the effect was not significant. The combined treatment provided the stronger cardioprotective effect (5278 ± 255 mmHg/s) than single therapies reaching significant difference compared to untreated HanSD controls; the peak value of pressure decline $[-(dP/dt)_{max}]$ exhibited similar changes (**Figure 4A**).

In untreated HanSD and TGR, MI induced significant systolic blood pressure reduction to 104.7 ± 2.6 and 97.0 ± 6.4 mmHg, respectively, compared to corresponding Sham (non-MI) animals (117.7 ± 3.7 and 139.6 ± 4.4 mmHg, respectively). Neither single

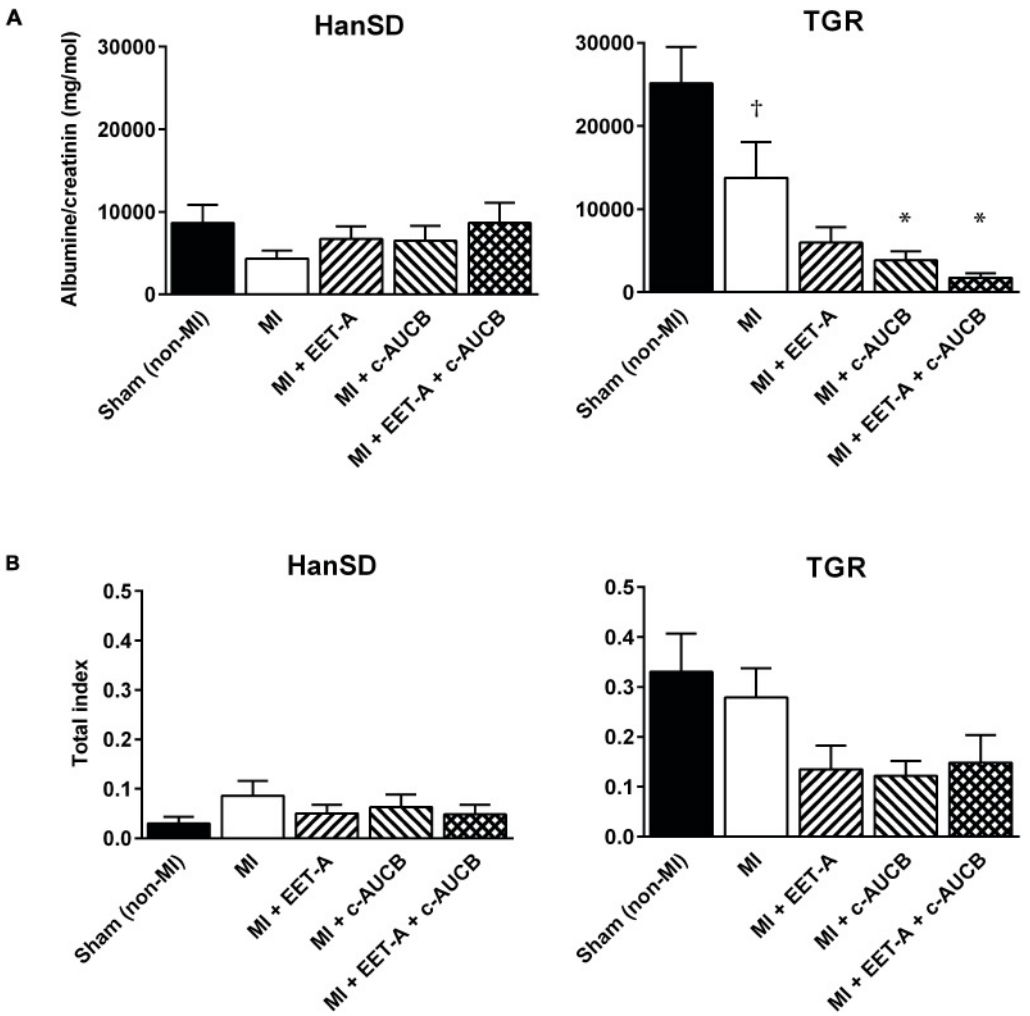

FIGURE 2 | Albumine/Creatinine clearance **(A)** and the total index of kidney injury (the sum of glomerulosclerosis and cortical tubulointerstitial injury; **B**) in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to sham operation (non-MI) or myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined for 5 weeks since 24 h after MI. Values are means ± SEM; †$P < 0.05$ MI vs. corresponding Sham (non-MI) group; *$P < 0.05$ vs. corresponding MI group.

nor combined treatment affected systolic blood pressure which varied between 102 and 108 mmHg in HanSD rats and 93–99 mmHg in TGR. In both strains, EET-based therapy did not significantly affect LV developed pressure in animals subjected to MI (**Figure 4B**). However, EET-A treatment, given alone or in combination, significantly reduced high LV end-diastolic pressure to 20.6 ± 2.5 and 19.4 ± 2.2 mmHg, respectively, compared with 30.5 ± 3.3 mmHg in untreated HanSD rats (**Figure 4C**). In TGR, neither single nor combined EET-based therapy affected post-MI LV dysfunction (**Figure 4**).

DISCUSSION

The main finding of the study is that the therapeutic administration of combined EET-based therapy after MI slowed down the progression of post-MI CHF in HanSD rats. As compared to normotensive strain, neither single nor combined treatment by EET analog and sEH inhibitor affected the progression of post-MI CHF in transgenic rats with Ang II-dependent hypertension. These effects were demonstrated by echocardiography as well as the direct LV catheterization.

In the present study, TGR were used as a well characterized experimental model of monogenetic hypertension of renal origin. The TGR strain [TGR(mRen2)27] was created by Mullins et al. (1990) as a rat model with an additional expression of the murine renin. Heterozygous TGR males reach maximum blood pressure at the age of 8–9 weeks (Lee et al., 1996). It has been demonstrated that TGR develop pathophysiological changes of the heart such as LV hypertrophy and myocardial fibrosis (Bachmann et al., 1992). With respect to the heart function, TGR exhibited unchanged (Habibi et al., 2011; Ma et al., 2012; Neckář et al., 2012; Červenka et al., 2015a; Kovács et al., 2016) or slightly lower LV systolic function (Whaley-Connell et al., 2007;

TABLE 2 | Echocardiographic parameters of left ventricle (LV) and heart rate (HR) in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to sham operation (non-MI) and myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined for 5 weeks since 24 h after MI.

	AWTd (mm)	LVDd (mm)	PWTd (mm)	AWTs (mm)	LVDs (mm)	PWTs (mm)	RWT (%)	HR (bpm)
HanSD								
Sham (non-MI)	2.21 ± 0.09	8.82 ± 0.20	2.28 ± 0.09	3.44 ± 0.11	5.27 ± 0.16	3.39 ± 0.10	51.1 ± 2.1	360 ± 14
MI	1.69 ± 0.08†	11.70 ± 0.17†	2.41 ± 0.10	1.66 ± 0.07†	10.37 ± 0.21†	2.88 ± 0.13†	35.1 ± 0.9†	327 ± 11
MI + EET-A	1.61 ± 0.05	11.24 ± 0.17	2.26 ± 0.07	1.62 ± 0.06	9.65 ± 0.17	2.92 ± 0.09	34.6 ± 0.7	347 ± 8
MI + *c*-AUCB	1.61 ± 0.05	11.13 ± 0.16	2.34 ± 0.10	1.62 ± 0.05	9.62 ± 0.20	2.95 ± 0.11	35.6 ± 1.2	355 ± 7
MI + EET-A + *c*-AUCB	1.60 ± 0.07	11.13 ± 0.24	2.16 ± 0.05	1.70 ± 0.12	9.50 ± 0.29*	2.83 ± 0.07	34.0 ± 1.1	356 ± 7
TGR								
Sham (non-MI)	2.64 ± 0.10	8.52 ± 0.17	2.57 ± 0.10	3.66 ± 0.14	5.44 ± 0.13	3.42 ± 0.10	65.1 ± 2.8	373 ± 13
MI	1.95 ± 0.08†	11.65 ± 0.29†	2.41 ± 0.09	2.03 ± 0.15†	10.35 ± 0.33†	3.16 ± 0.11	35.0 ± 1.6†	353 ± 11
MI + EET-A	1.88 ± 0.08	11.19 ± 0.30	2.41 ± 0.05	1.93 ± 0.13	9.90 ± 0.35	3.17 ± 0.06	38.1 ± 2.2	351 ± 15
MI + *c*-AUCB	1.84 ± 0.06	11.54 ± 0.17	2.40 ± 0.09	1.96 ± 0.14	10.28 ± 0.24	3.00 ± 0.09	35.3 ± 1.0	343 ± 10
MI + EET-A + *c*-AUCB	1.85 ± 0.08	11.47 ± 0.16	2.43 ± 0.11	1.94 ± 0.18	10.30 ± 0.16	3.10 ± 0.14	35.4 ± 1.7	321 ± 16

*AWTd, diastolic anterior wall thickness; LVDd, diastolic LV diameter; PWTd, diastolic posterior wall thickness; AWTs, systolic anterior wall thickness; LVDs, systolic LV diameter; PWTs, systolic posterior wall thickness; RWT, relative wall thickness; bpm, beats per minute. Values are means ± SEM; †P < 0.05 MI vs. corresponding Sham (non-MI) group; *P < 0.05 vs. corresponding MI group.*

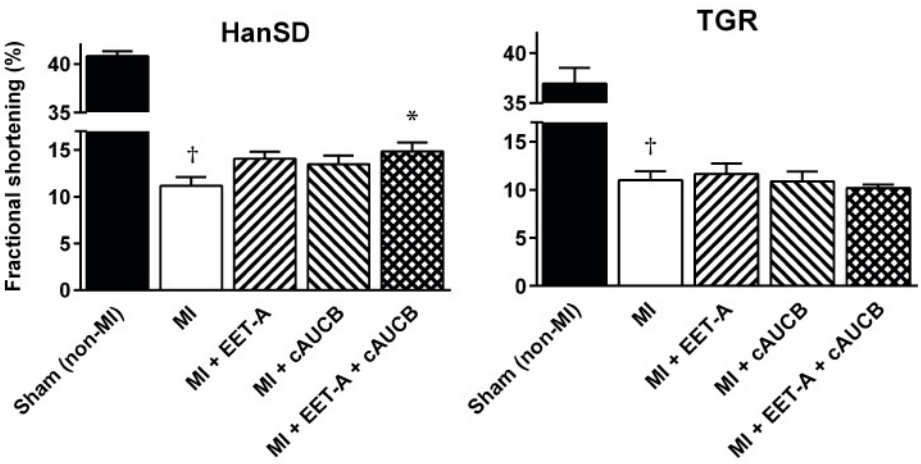

FIGURE 3 | Fractional shortening in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to Sham (non-MI) operation or myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined for 5 weeks since 24 h after MI. Values are means ± SEM; †$P < 0.05$ MI vs. corresponding Sham (non-MI) group; *$P < 0.05$ vs. corresponding MI group.

De Mello et al., 2013), depending on measured heart parameters and used methods. Further, it has been shown that TGR have increased mortality compared to normotensive HanSD rats when subjected to volume overload (Červenka et al., 2015a,b; Kala et al., 2018). Finally, there is a single paper dealing with the progression of post-MI CHF in TGR (Connelly et al., 2013). Using TGR females, it demonstrated the LV function impairment after MI comparable to our present study. It also showed that combined pharmacological treatment with angiotensin converting enzyme and direct renin inhibition blunted the progression of post-MI CHF.

The present study follows on from our recent report (Červenka et al., 2018) which analyzed the preventive effect of the same EET-based therapy on the acute cardiac ischemic tolerance in HanSD rats and TGR. We demonstrated that EET-A and *c*-AUCB, given alone or combined before MI (two-week treatment), did not affect the infarct size in both strains and had no additional effects on hearts of HanSD rats. However, both single and combined EET-based therapy lowered high blood pressure, decreased LV hypertrophy and reduced the increased incidence of ischemia-induced ventricular fibrillation in hypertensive TGR (Červenka et al., 2018). These findings provided the impetus to conduct the current experimental study to determine effects of EET-A and *c*-AUCB, given alone or combined after MI.

Here we examined effects of single and combined EET-A treatment on the progression of post-MI CHF. The third generation of orally active EET agonist analogs, including EET-A, demonstrated great potential for therapy of cardiovascular and kidney diseases in rat and mouse models. Indeed, the most promising compounds (EET-A and EET-B) were validated as powerful 14,15-EET analogs (Falck et al., 2014; Khan

TABLE 3 | Doppler echocardiography of mitral and pulmonary artery flow in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to sham operation (non-MI) and myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined for 5 weeks since 24 h after MI.

	Mitral flow					Pulmonary artery flow			
	Vm_{max} ($m.s^{-1}$)	FT (ms)	IVCT (ms)	ET (ms)	IVRT (ms)	Vpa_{max} ($m.s^{-1}$)	Vpa_{mean} ($m.s^{-1}$)	AT (ms)	ETpa (ms)
HanSD									
Sham (non-MI)	1.11 ± 0.04	64.1 ± 3.6	13.9 ± 1.2	67.9 ± 4.4	23.7 ± 1.8	1.06 ± 0.05	0.46 ± 0.02	27.5 ± 1.5	95.1 ± 3.0
MI	1.32 ± 0.04†	45.2 ± 1.7†	49.2 ± 5.3†	61.5 ± 2.0	29.5 ± 1.8	0.77 ± 0.05†	0.31 ± 0.02†	25.4 ± 1.6	92.1 ± 2.2
MI + EET-A	1.29 ± 0.04	49.6 ± 1.6	40.0 ± 5.5	63.7 ± 1.8	25.5 ± 2.2	0.92 ± 0.04	0.39 ± 0.02*	28.0 ± 1.4	91.1 ± 1.2
MI + *c*-AUCB	1.31 ± 0.06	46.6 ± 1.8	30.5 ± 3.2*	59.9 ± 3.5	30.6 ± 2.7	0.89 ± 0.04	0.37 ± 0.02	27.5 ± 1.9	89.5 ± 2.4
MI + EET-A + *c*-AUCB	1.17 ± 0.05	54.0 ± 2.0*	31.4 ± 3.7*	57.9 ± 1.9	26.3 ± 1.4	0.92 ± 0.04	0.39 ± 0.02*	27.1 ± 1.4	90.7 ± 1.3
TGR									
Sham (non-MI)	1.20 ± 0.04	59.5 ± 3.0	14.6 ± 1.6	63.8 ± 2.0	21.4 ± 2.6	1.08 ± 0.03	0.47 ± 0.02	28.2 ± 1.3	89.8 ± 1.7
MI	1.51 ± 0.06†	44.8 ± 1.9†	35.9 ± 4.7†	59.1 ± 3.8	32.0 ± 4.3	0.80 ± 0.06†	0.32 ± 0.03†	25.2 ± 1.4	88.1 ± 1.5
MI + EET-A	1.36 ± 0.06	47.0 ± 1.3	41.3 ± 7.3	61.2 ± 4.8	31.3 ± 7.2	0.89 ± 0.05	0.37 ± 0.03	25.4 ± 0.7	87.7 ± 1.5
MI + *c*-AUCB	1.38 ± 0.06	47.0 ± 2.5	46.1 ± 5.8	64.8 ± 2.9	20.7 ± 1.7	0.83 ± 0.04	0.34 ± 0.03	26.3 ± 1.3	90.4 ± 2.0
MI + EET-A + *c*-AUCB	1.27 ± 0.09	43.1 ± 3.5	56.2 ± 9.4	61.0 ± 4.7	29.0 ± 3.8	0.75 ± 0.04	0.30 ± 0.03	26.7 ± 1.7	91.2 ± 1.3

*Vm_{max}, left ventricle filling peak velocity; FT, filling time; IVCT, isovolumic contraction time; ET, left ventricle ejection time; IVRT, isovolumic relaxation time; Vpa_{max}, peak velocity; Vpa_{mean}, mean velocity; AT, acceleration time; ETpa, pulmonary artery ejection time. Values are means ± SEM; †$P < 0.05$ MI vs. corresponding Sham (non-MI) group; *$P < 0.05$ vs. corresponding MI group.*

et al., 2014; Campbell et al., 2017). It has been shown that these novel and orally active EET analogs provided heart and kidney protection comparable with that of native EETs (Skibba et al., 2017; Neckář et al., 2018). Previously, Imig's group demonstrated that EET-A markedly reduced cisplatin-induced nephrotoxicity and mitigated radiation nephropathy in rats (Khan et al., 2013; Hye Khan et al., 2016). EET-A also ameliorated the deleterious effects of high fat diet-induced metabolic abnormalities in obese mice (Singh et al., 2016). In the same mouse model (db/db mice), EET-A treatment improved LV systolic function (Cao et al., 2017). Recently, we have shown that the continuous treatment by another 14,15-EET analog EET-B before and after MI reduced post-MI mortality and the progression of LV systolic dysfunction in spontaneously hypertensive rats (Neckář et al., unpublished) Compared to predominant EET analogs-mediated protection against end-organ injury, their antihypertensive action is rather inconsistent; rodent models with various genetic background of hypertension differ in their sensitivity to EET-based therapy. Hence, EET-A reduced blood pressure in various forms of Ang II-dependent models of hypertension in rats (Neckář et al., 2012; Hye Khan et al., 2014; Červenka et al., 2018) and in mice with high fat diet-induced obesity (Singh et al., 2016). On the other hand, EET-A or EET-B did not exhibit any antihypertensive action in Dahl salt-sensitive rats, Goldblatt hypertensive rats, Cyp1a1-Ren-2 transgenic rats, and in spontaneously hypertensive rats (Hye Khan et al., 2013; Alánová et al., 2015; Jíchová et al., 2016; Neckář et al., unpublished). Therefore, it seems that the protective action of EET analogs against end-organ injury is independent of blood pressure reduction in hypertensive animal models.

The progression of post-MI CHF in the present study was associated with decreased albuminuria and the total index of kidney injury in untreated TGR compared to Sham (non-MI) controls. We speculate that these findings can reflect blood pressure reduction after MI. Indeed, it is well known that post-MI CHF decreases blood pressure in hypertensive rats due to insufficient myocardial function (Nishikimi et al., 1995; Mori et al., 1998; Wiemer et al., 2001) which can result in reduced kidney injury. Further, EET-based therapy almost eliminated albuminuria and decreased kidney injury score in TGR. This finding is not surprising because sEH inhibitors and EET analogs, respectively, represent promising and powerful therapies to prevent the progression of various chronic kidney diseases to renal failure (Imig, 2015; Fan and Roman, 2017). Similarly, preventive treatment of TGR by the same EET-based therapy before MI resulted in kidney protection (Červenka et al., 2018). The absence of any differences in other urinary markers of renal injury suggests that post-MI progression of CHF did not substantially damage kidney in both strains.

Previously, it has been demonstrated that acute exogenous administration of EETs or inhibition of sEH attenuated the increase of endothelial cell permeability and lung injury after acute ischemia/reperfusion or lipopolysaccharides administration (Townsley et al., 2010; Chen et al., 2015; Tao et al., 2016). Further, long-lasting sEH inhibitor treatment reduced bleomycin-induced pulmonary injury (Dong et al., 2017). It seems that EETs can protect against various lung diseases associated with inflammation and oxidative stress like asthma and chronic obstructive pulmonary disease (Yang et al., 2015, 2017). In line with these observations, EET-based therapy moderately limited CHF-induced lungs edema and improved PA flow in HanSD rats in the present study.

It has been shown that chronic treatment with sEH inhibitors reduced the progression of post-MI LV systolic dysfunction in normotensive animals (Li et al., 2009; Merabet et al., 2012;

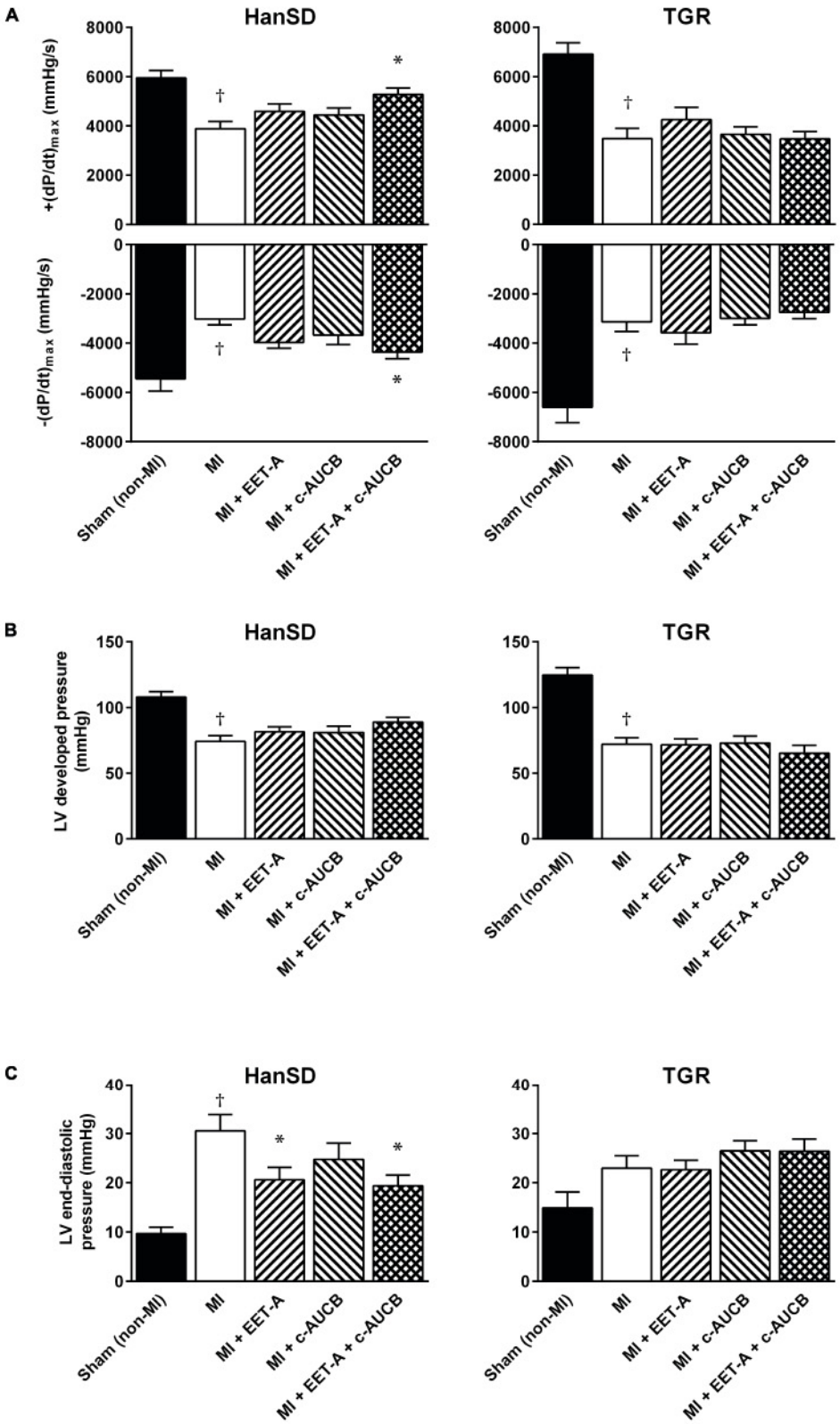

FIGURE 4 | Left ventricle peak rates of pressure development and fall **(A)**, developed pressure **(B)**, and end-diastolic pressure **(C)** in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to Sham (non-MI) operation or myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined for 5 weeks since 24 h after MI. Values are means ± SEM; †$P < 0.05$ MI vs. corresponding Sham (non-MI) group; *$P < 0.05$ vs. corresponding MI group.

Kompa et al., 2013; Sirish et al., 2013). The beneficial effect of different sEH inhibitors was associated with increased EETs levels (Li et al., 2009; Sirish et al., 2013). Similarly, *c*-AUCB (given alone or in combination with EET-A) increased the myocardial concentration of endogenous EETs in both HanSD rats and TGR (Červenka et al., 2018). Moreover, Merabet et al. (2012) demonstrated that the cardioprotective action of sEH treatment was abolished by co-administration with an inhibitor of cytochrome P450 epoxygenase, the enzyme producing EETs from arachidonic acid. Therefore, EETs can play a role in the progression of post-MI CHF in normotensive rats, which is in line with our present study.

The effect of sEH inhibitors on the progression of CHF associated etiologies other than ischemic heart disease is inconsistent. Indeed, it has been reported that other sEH inhibitors such as AEPU, AUDA and TPPU reduced the development of cardiac hypertrophy and diminished adverse cardiac remodeling in normotensive mice subjected to pressure overload due to thoracic aortic constriction (Xu et al., 2006; Sirish et al., 2013). On the other hand, another sEH inhibitor GSK2256294 did not reverse LV dysfunction induced by pressure overload in both mice and rats, in spite of the fact that the increased an EETs-to-DHETs ratio was observed (Morgan et al., 2013). Similarly, *c*-AUCB did not alter LV contractility in hypertensive TGR and Fawn-hooded rats as well as normotensive HanSD and Fawn-hooded low-pressure rats subjected to volume overload (Červenka et al., 2015a,b; Vacková et al., 2019).

The overall biology of both mimics of epoxy fatty acids (EpFA) and sEH inhibitors are anticipated to be similar and in some cases additive. An intrinsic problem with sEH inhibitors is that they can only preserve the EpFA that are present. This has the advantage of making it difficult to over dose, but a disadvantage is that the sEH inhibitors cannot correct for abnormally low levels of EpFA. The mimics on the other hand do not require endogenous production of EpFA for their biological activity. However, the mimics only mimic a single isomer of the complex array of EpFAs present in an organism while sEH inhibitors to some degree preserve all EpFA. The preservation is likely to be related to the endogenous ratios of endogenous EpFA. Thus each pharmaceutical approach offers endogenous advantages and limitations. One can anticipate situations where the effect of the two drug classes would likely be additive. As individual EpFA mimics and sEH inhibitors are selected for development each will present unique pharmacokinetic parameters which will offer specific limitations and assets (Shen and Hammock, 2012).

CONCLUSION

In conclusion, our results showed that combined EET-based therapy reduced the progression of post-MI CHF in normotensive HanSD rats. Even though they exhibited renoprotective action, neither single nor combined treatment by EET-A and *c*-AUCB affected the extent of post-MI CHF in *Ren-2* transgenic rats with Ang II-dependent form of hypertension. Cardioprotective efficacy of EET-based therapy against the progression of CHF varies depending on experiment model and protocol, and associated comorbidities.

DATA AVAILABILITY

All datasets generated for this study are included in the manuscript and/or the **Supplementary Files**.

AUTHOR CONTRIBUTIONS

JN primarily conceived and designed the study, performed experiments, and wrote the manuscript. JH performed experiments, analyzed and interpreted the data, and wrote the manuscript. BH, SH, JI, and JF designed and synthesized *c*-AUCB and EET-A. FP, ZH, SK, ZVa, ZVe, FA, JV, LČ, 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.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fphar.2019.00159/full#supplementary-material

FIGURE S1 | Plasma concentration of monocyte chemoattractant protein-1 (MCP-1) at the end of study in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to Sham (non-MI) operation or myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined for 5 weeks since 24 h after MI. Values are means ± SEM.

FIGURE S2 | Urinary sodium **(A)**, potassium **(B)** and cystatin c **(C)** excretion at the end of study in Hannover Sprague-Dawley (HanSD) and *Ren-2* transgenic rats (TGR) subjected to Sham (non-MI) operation or myocardial infarction (MI) and treated with epoxyeicosatrienoic acid analog (EET-A) or soluble epoxide hydrolase inhibitor (*c*-AUCB), given alone or combined for 5 weeks since 24 h after MI. Values are means ± SEM.

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