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Soluble epoxide hydrolase gene deletion reduces survival after cardiac arrest and cardiopulmonary resuscitation[☆]

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Abstract

Summary—The P450 eicosanoids epoxyeicosatrienoic acids (EETs) are produced by cytochrome P450 arachidonic acid epoxygenases and metabolized through multiple pathways, including soluble epoxide hydrolase (sEH). Pharmacological inhibition and gene deletion of sEH protect against ischemia/reperfusion injury in brain and heart, and against hypertension-related end-organ damage in kidney. We tested the hypothesis that sEH gene deletion improves survival, recovery of renal function and pathologic ischemic renal damage following transient whole-body ischemia induced by cardiac arrest (CA) and resuscitation. Mice with targeted deletion of sEH (sEH knockout, sEHKO) and C57Bl/6 wild-type control mice were subjected to 10-min CA, followed by cardiopulmonary resuscitation (CPR). Survival in wild-type mice was 93% and 80% at 10 min and 24 h after CA/CPR ($n = 15$). Unexpectedly, survival in sEHKO mice was significantly lower than WT. Only 56% of sEHKO mice survived for 10 min ($n = 15$, $p = 0.014$ compared to WT) and no mice survived for 24 h after CA/CPR ($p < 0.0001$ versus WT). We conclude that sEH plays an important role in cardiovascular regulation, and that reduced sEH levels or function reduces survival from cardiac arrest.

Keywords

Cardiac arrest; CPR; Ischemia; Blood pressure; Epoxyeicosatrienoic acids; P450 epoxygenase; Soluble epoxide hydrolase; EPHX2

Introduction

The P450 epoxygenase pathway metabolizes arachidonic acid (AA) into biologically active eicosanoids referred to as epoxyeicosatrienoic acids (EETs).¹ In the systemic circulation, EETs are primarily produced by vascular endothelium, where they serve as an endothelium-derived hyperpolarizing factor (EDHF).² As such, EETs play an important role in regulating tissue perfusion in several organs, including heart, brain and kidney. However, EETs are short-lived, mainly due to metabolic conversion by soluble epoxide hydrolase (sEH) into dihydroxyeicosatrienoic acids (DHETs).³ Recent reports suggest that sEH inhibition is

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protective against cardiovascular disease, including hypertension-related end-organ damage.^{4,5} In agreement with these reports, we have also shown that sEH inhibition is protective against stroke-related ischemic brain damage.⁶ Furthermore, sEH gene deletion in sEH knockout (sEHKO) mice renders these mice resistant to angiotensin II-induced hypertension.⁷ More recently, using an isolated perfused heart, Seubert et al. demonstrated that sEHKO mice exhibit improved ventricular function after myocardial ischemia.⁸ However, the impact of sEH gene deletion on survival and end-organ tissue damage in an in vivo model of whole-body ischemia remains unknown. Furthermore, vasodilation, while beneficial in focal ischemia, could be detrimental during cardiac resuscitation as it would lower the coronary perfusion pressure and critically compromise myocardial blood flow. Therefore, in the current study, we used an in vivo mouse model of cardiac arrest (CA) followed by cardiopulmonary resuscitation (CPR) to test the hypothesis that sEHKO mice demonstrate improved survival, renal functional recovery and attenuated pathologic ischemic renal damage. We here report the very significant, yet surprising, finding that sEH gene deletion, which protects against ischemic damage in an isolated heart preparation, impedes CPR and worsens survival after CA in vivo. This is a novel finding, with important clinical implications related to understanding mechanisms and developing new therapeutic agents for the prevention of and the facilitation of recovery from cardiac arrest.

Materials and methods

The study was conducted in accordance with the National Institute of Health guidelines for the care and use of animals in research and protocols were approved by the institutional animal care and use committee. The sEHKO strain was obtained from Dr. Frank Gonzalez at the National Institutes of Health, where it was originated. Gene disruption strategy and phenotype are described elsewhere.⁷ The strain has been backcrossed to C57Bl/6 for at least six generations; and therefore, homozygous sEHKO mice were compared in our study to wild-type (WT) C57BL/6 mice obtained from The Jackson Laboratories. Homozygous sEHKO mice are viable, fertile, normal in size and do not display any gross physical or behavioral abnormalities. Mouse genotype was confirmed by PCR as previously described.⁷ Adult male C57BL/6 mice (WT, 6–8 weeks of age, $n = 15$) and age- and weight-matched sEHKO mice ($n = 15$) were subjected to cardiac arrest (CA), as previously described.^{9–15} Briefly, in random order with respect to their strain, mice were removed from their home cages, and anesthesia was induced with 4% isoflurane, and subsequently maintained with 1–2% isoflurane in air/oxygen mixture. Mice were weighed, positioned on the operating table and mechanically ventilated after tracheal intubation with a 22 gauge catheter. Body temperature was monitored with a rectal probe and maintained at 37 ± 0.5 °C with a heating lamp and warm pad.

Using sterile technique, a PE-10 catheter was inserted into the right jugular vein for intravenous (i.v.) drug infusion. EKG was monitored with subdermal electrodes, and arterial blood pressure was monitored using a femoral arterial catheter in a cohort of 6 animals (3 WT and 3 sEHKO mice). As illustrated schematically in Figure 1, CA was induced with 40 μ L iced 0.5 M KCl i.v. and confirmed by ECG and, when present, arterial blood pressure measurement. The ECG rhythm during CA is asystole, confirmed in two leads. In previous experimentation in our lab, we have observed excellent concordance between pulseless hypotension and ECG asystole. Ventilation was stopped and the tracheal tube disconnected from the ventilator. After 9 min and 30 s of normothermic CA with no ventilation, the tracheal tube was reconnected to the ventilator, and hyperventilation at 120% of pre-arrest rate was initiated using 100% O₂. At 10 min, chest compressions were initiated at a rate of 300 bpm, and epinephrine (8–15 mcg in 0.5–1 mL normal saline) was administered intravenously in divided doses. Epinephrine (adrenaline) is delivered at a uniform rate of 0.225 μ g/17 μ L/s (7.5 μ g in the first 30 s of CPR). If further epinephrine is used, it is at the investigator's discretion, governed largely by the length of resuscitation. Chest compressions are achieved using the investigator's index finger. Tapping

the mouse chest at 300 bpm requires extensive training, but is facilitated by the fact that the rate is visible on ECG as motion artifact. CPR was discontinued upon return of spontaneous circulation (ROSC) as observed on the ECG, or when 4 min of CPR had passed without ROSC. On ROSC, cardiac contractions are visible in the anterior chest wall. Within a minute of ROSC, mucous membrane color transitions from grey to pink. ECG and temperature were closely monitored for 10 min after ROSC. Animals were extubated when spontaneous respiratory rate was greater than 60/min, usually between 12 and 18 min after ROSC. The jugular catheter was removed, hemostasis obtained, and animals returned to cages. The recovery cage was placed on a warming mat set at 37 °C to maintain normothermia in the post-arrest period. Animals are observed continuously over the first hour of resuscitation. Extensive monitoring; approximately every hour, continues until the end of the working day, and resumed in the morning for surviving animals. Surviving animals were deeply anesthetized at 24 h after CA, and tissue fixed through transcardiac perfusion with 4% paraformaldehyde in saline for subsequent renal histological analysis.

Statistical analysis

Data analysis was performed with Prism 5.0 statistical software. The primary outcome was survival. Two group comparisons of survival and other outcomes were made using unpaired Student's *t*-test. Statistical significance was set at $p < 0.05$. Data are reported as mean $\pm$ S.E.M.

Results

There were no significant differences between WT and sEHKO mice with regard to pre-arrest body weight, baseline or mean intra-arrest rectal temperature (Table 1, $n = 15$ per group). Both strains of mice were subjected to identical protocol of 10-min normothermic cardiac arrest, followed by cardiopulmonary resuscitation (CPR) under isoflurane anesthesia. In WT mice, restoration of spontaneous circulation (ROSC) was observed in all mice (15/15, 100%), which required 12.0 ± 0.5 mcg of epinephrine and 110.3 ± 8.2 s of CPR, resulting in 93% and 80% survival at 10 min and 24 h after CA/CPR (14 and 12 survivors out of 15 mice, respectively). Because epinephrine was infused at a fixed rate and fixed concentration, sEHKO mice received larger fluid volumes during resuscitation compared to WT mice (0.8 ± 0.03 mL over 48 s in WT mice compared to 1.7 ± 0.3 over 104 s in sEHKO mice). Unexpectedly, however, only 60% (9/15) of sEHKO mice exhibited signs of ROSC. They required significantly more epinephrine (26.0 ± 4.9 mcg, $n = 12$, $p = 0.003$, Figure 2), and longer CPR time (163.3 ± 28.9 s, $n = 12$, Figure 3) compared to WT mice, although the difference in CPR time was not statistically significant ($p = 0.06$). More importantly, survival was markedly lower in sEHKO compared to WT mice. Only 46% of sEHKO mice survived for 10 min (7 out of 15), and no sEHKO mice survived 24 h after CA/CPR ($n = 15$, Figure 4). Most of the 10-min survivors expired within 2 h post-arrest. In a separate cohort of WT and sEHKO mice, mean arterial blood pressure (MAP) was monitored during cardiac arrest and for 10 min after resuscitation (Figure 5). In WT mice, MAP recovered to 103 ± 11 mmHg by 1 min, peaked at 112 ± 3 mmHg by 3 min, and reached 75 ± 10 at 10 min after CA/CPR. Arterial blood pressure recovery was delayed in sEHKO mice, reaching 86 ± 7 mmHg at 1 min, peaking at 107 ± 3 mmHg by 5 min, dropping to 62 ± 8 mmHg at 10 min after CA/CPR. The differences in blood pressure did not achieve statistical significance at any time point over the 10-min period after ROSC.

Discussion

The main finding of our study is that sEH gene deletion renders mice refractory to cardiopulmonary resuscitation (CPR) after cardiac arrest (CA). The sEHKO mice required significantly higher doses of epinephrine and longer CPR time, demonstrated delayed blood pressure recovery after CPR and suffered significantly higher mortality compared to wild-type

control mice. Our findings suggest that sEH plays an important role in recovery from cardiac arrest, likely due to its EETs-metabolizing function.

Soluble epoxide hydrolase (sEH) is a major pathway for the metabolic conversion of P450 eicosanoids epoxyeicosatrienoic acids (EETs). Inhibition of sEH, which increases EETs bioavailability by decreasing their breakdown, has been shown to be protective against focal cerebral ischemia.⁶ Similarly, ventricular function after myocardial ischemia was preserved in isolated heart preparation from sEHKO compared to WT mice. Finally, sEH inhibition attenuated hypertension-related renal and brain damage.^{5,16} The protective effects of sEH inactivation were presumably due to the beneficial properties of EETs, which include vasodilation,¹⁷ suppression of inflammation,¹⁸ prevention of platelet aggregation¹⁹ and protection against apoptotic²⁰ and ischemic^{21,22} cell death. Based on these studies, it was reasonable to hypothesize that sEH gene disruption would confer protection against tissue injury induced by whole-body ischemia-reperfusion. Therefore, in the current study, we set out to determine the impact of sEH gene deletion on kidney damage after CA/CPR. However, we were unable to assess the extent of end-organ damage in sEH gene disrupted mice because of the unexpected substantial mortality suffered by sEH knockout mice after CA/CPR.

Although our available data does not allow us to reach a definitive conclusion as to the precise cause of death in sEHKO mice, we speculate that the cause of death is linked to increased EETs, since EETs metabolism is the best characterized and well known function of sEH, and because most of the reported phenotypic features of sEHKO can be explained by higher levels of EETs in these mice. Furthermore, because the best known and well characterized biological property of EETs is vasodilation, we examined the possibility that increased mortality in sEHKO mice was attributed to vascular collapse and inability to restore blood pressure, due to unopposed vasodilation by EETs. Our data suggest that arterial blood pressure recovery might be slower in sEHKO compared to WT mice, requiring higher doses of epinephrine and longer CPR time, although the difference in blood pressures between the two groups was not statistically significant at any time point. As mentioned above, EETs are known vasodilators^{23,24} in multiple vascular beds, including coronary and cerebral circulation,²⁵ and sEHKO mice are resistant to angiotensin- and salt load-induced hypertension.⁷ Therefore, it is possible that sEH gene disruption confers either a baseline state of increased arteriolar vasodilation relative to wild-type, or a resistance to the effects of exogenous vasoconstrictors, or both. In our study, however, we did not observe a difference in baseline mean arterial blood pressure (MAP) between WT and sEHKO mice. This is in agreement with one published study²⁶, but in contrast to another, which showed a significant difference in baseline MAP between WT and sEHKO mice⁷. It is unlikely that increased mortality in sEHKO mice is related to cardiac dysfunction, since sEHKO mice have similar cardiac mass and myocardial function as WT mice⁸. Nevertheless, our data does not rule out the possibility that a primary cardiac dysfunction may underlie the increased mortality in sEHKO mice. Increased mortality in sEHKO mice may be related to other yet unrecognized effects of EETs. For example, sEH inhibition has been shown to increase pulmonary vascular resistance,²⁷ which, if it takes place in sEHKO mice after CA, would lead to pulmonary hypertension and right ventricular failure, and would impede resuscitation. Finally, it is also possible that blood vessels from sEHKO mice are resistant to the pressor effect of vasoconstrictors, including epinephrine.

The following limitations should be considered when interpreting our data. First, we did not measure cardiac performance directly, which would have definitively ruled out myocardial dysfunction in sEHKO mice. Second, because mortality in sEHKO mice was 100%, we were unable to proceed with planned histological analyses, which would have answered the question of whether peripheral tissue is protected from ischemia/reperfusion damage in the sEHKO mice. Finally, the cardiac arrest model is a severe whole-body ischemia model, in which multiple organs are affected, making it difficult to identify the primary cause of death.

Nonetheless, significant claims have been made for organ protective effects of EETs and sEH inhibition and gene disruption, based on in vitro and focal ischemia models. We believe this is the first demonstration of a significant deleterious effect of sEH gene disruption in a clinically relevant model of cardiac arrest-induced global ischemia.

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Figure 1.

Cartoon depicting key events and procedures of the mouse model of normothermic cardiac arrest. Pre-arrest: on the EKG, sinus rhythm is observed (normal HR $\approx$ 300). The MAP rises to normal (60 mmHg) in the pre-arrest period as anesthetic is titrated down from induction. Intra-arrest: cardiac arrest is induced with 40 μ L of KCl injected via the jugular catheter and asystole is observed on the EKG. In animals with a femoral catheter, a low, nonpulsatile arterial pressure is observed. CPR: motion artifact from CPR is visible on the ECG, and can be used to keep a constant rate. Epinephrine (see text for dose) is delivered via the jugular catheter. In normal animals, ROSC is observed within 120 s. If an arterial catheter is present, a pulsatile pressure waveform is observed and the MAP rises to above pre-arrest baseline. On the ECG, ROSC is seen as isolated QRS complexes coalescing into sinus tachycardia. Post-arrest: the post-arrest rhythm is sinus tachycardia. Normothermia continues to be maintained. MAP declines to baseline in normal animals.

Figure 2.

Total epinephrine dose required for resuscitation. Epinephrine was delivered in divided doses while chest compressions were underway. sEHKO mice required significantly more epinephrine than WT mice (26.0 ± 4.9 mcg, $n = 12$, as compared to 12.0 ± 0.5 mcg, $n = 15$, respectively, mean $\pm$ S.E.M.), $*p = 0.003$.

Figure 3.

Time to restoration of spontaneous circulation (ROSC). Time was measured from initiation of CPR to ROSC. Chest compressions were delivered at a rate of 300/min at the same pressure by the same investigator. Surviving sEHKO mice required longer CPR time than WT mice (163.3 ± 28.9 ($n = 12$) compared to 110.3 ± 8.2 ($n = 15$) seconds, respectively, although the difference was not statistically significant, $p = 0.06$).

Figure 4.

Percent survival at 10 min and 24 h after CA/CPR. Each bar represents 15 animals. Bar portion above the X-axis represents survival, portion below represents mortality. sEHKO mortality was 44% and 100% at 10 min and 24 h compared to 7% and 20% in WT, respectively, * $p=0.014$, ‡ $p<0.0001$.

Figure 5.

Mean arterial blood pressure (MAP) in the 18 min period after CA/CPR. sEHKO mice recover from cardiac arrest with lower 1-min MAP (86 ± 7 mmHg vs. 103 ± 11 mmHg in WT), achieve lower and more delayed peak (107 ± 3 mmHg at 5 min vs. 112 ± 3 mmHg at 3 min in WT) and have lower MAP at 10 min post-ROSC (62 ± 8 mmHg vs. 75 ± 9 mmHg, $n = 3$ per group, mean $\pm$ S.E.M.) and 18 min post-ROSC (48 ± 0 mmHg vs. 73 ± 9 mmHg, $n = 3$ per group, mean $\pm$ S.E.M.).

Table 1

Baseline values in WT and sEHKO mice. There were no significant differences between the two strains in body weight, baseline MAP, or baseline or intra-arrest temperature

Strain	Weight (g, mean $\pm$ S.E.M., $n = 15 \text{ g}^{-1}$)	Baseline MAP (mmHg, mean $\pm$ S.E.M., $n = 3 \text{ g}^{-1}$)	Baseline temperature ($^{\circ}\text{C}$, mean $\pm$ S.E.M., $n = 15 \text{ g}^{-1}$)	Mean arrest temperature ($^{\circ}\text{C}$, mean $\pm$ S.E.M., $n = 15 \text{ g}^{-1}$)
WT	24.1 $\pm$ 1.05	90.0 $\pm$ 4.7	36.6 $\pm$ 0.16	37.0 $\pm$ 0.02
sEHKO	21.5 $\pm$ 0.64	74.0 $\pm$ 0.6	36.0 $\pm$ 0.22	36.7 $\pm$ 0.21