Inhibition of Soluble Epoxide Hydrolase Reduces Infarct After Myocardial Ischemia-Reperfusion In Vivo

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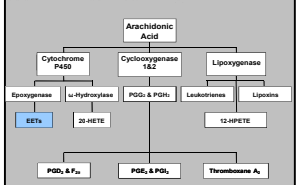
ABSTRACT – A301

Introduction. Soluble epoxide hydrolase (sEH) is the primary enzyme that metabolizes epoxyeicosatrienic acids (EETs), polyunsaturated fatty acids derived from arachidonic acid. EETs are released during ischemia and are linked to cardioprotection against ischemia-reperfusion. In vivo, EETs are rapidly broken down via sEH. Functional recovery is improved in isolated hearts from sEH knockout mice after global ischemia[1]. Pharmacological inhibition of sEH should augment endogenously released EETs, and result in cardioprotection against myocardial ischemia-reperfusion injury. **Hypothesis.** We tested the hypothesis that pharmacologic sEH inhibition reduces myocardial ischemic injury in vivo. **Method.** We subjected male C57BL\6J mice to 40 min of left coronary artery occlusion (LCA) and 2 h reperfusion. AUDA-BE (10 mg/kg), a sEH inhibitor, or vehicle (sesame oil) was given by intraperitoneal injection 30 min prior to LCA occlusion. Area-at-risk (AAR) and infarct size (I) were assessed using fluorescent microspheres and triphenyl tetrazolium chloride staining. Infarct size is expressed as I/AAR (mean±SEM). **Results.** I/AAR was significantly smaller in AUDA-BE treated animals compared to vehicle control (30±5% versus 46±3%, p<0.01). AAR/biventricular volume was similar in both groups (28±2% versus 33±1%, ns). **Conclusion.** Inhibition of sEH prior to ischemia-reperfusion elicits cardioprotection in vivo. Protection is likely due to increased endogenous myocardial EETs levels. Pharmacological inhibition of sEH may serve as a novel therapeutic option for cardioprotection against myocardial ischemia-reperfusion injury during coronary revascularization or organ preservation for cardiac transplantation. Support: VA Merit Review 317 (DMVW) and RO1 NS44313 (NJA).

CONCEPT

Arachidonic Acid Pathway



BACKGROUND

Arachidonic acid (AA) is released from membrane phospholipids in response to a variety of pathophysiologic and pharmacologic stimuli, including myocardial ischemia. Free AA is metabolized by three pathways: cyclooxygenase, lipoxygenase and cytochrome P450 monooxygenase (CYP). CYP metabolizes AA to 4 biologically active eicosanoids (epoxyeicosatrienoic acids EET): 5,6-EET; 8,9-EET; 11,12-EET and 14,15-EET. EETs have anti-inflammatory and anti-thrombotic effects, are coronary vasodilators, and reduce myocardial ischemic injury (1-4). The biological activity of EETs is rapidly terminated by hydration into less active dihydroeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH). Intracoronary infusion of sEH inhibitor AUDA prior to ischemia in dogs reduced infarct size (5) and functional recovery is improved in isolated hearts from sEHKO mice after global ischemia (6).

HYPOTHESIS

Pharmacologic inhibition of sEH prior to reperfusion reduces myocardial ischemic injury in vivo via 14,15-EET mediated pathway.

METHOD

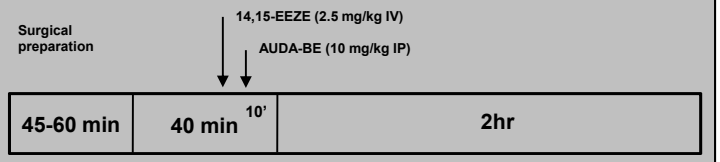
Animals:

Male C57BL\6J mice received treatment in compliance with the Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Research, National Research Council; National Academy Press, 1996) and with IACUC approval.

Regional Myocardial Ischemia-Reperfusion Injury.

Mice were anesthetized with isoflurane and intubated with a 20G plastic IV catheter and ventilated. ECG and rectal temperature were monitored. The animals were positioned in a right lateral decubital position and rectal temperature were maintained at 37ºC. A PE-10 catheter was inserted into the jugular vein for drug infusion. A left-sided thoracotomy was performed in the 4th intercostal space. A ligature was placed around the Left Coronary Artery (LCA). The LCA was occluded for 40 min; occlusion was confirmed with persistent ECG changes during occlusion and visual paling of the left ventricle (LV). After 40 min the snare was released and reperfusion was confirmed with visual hyperemia of the LV and return of the ECG to baseline. After 2 hours of reperfusion the LCA was re-occluded. Fluorescent microspheres were infused via needle puncture of the LV apex and the heart was excised. The ventricles were sliced into seven sections (d=1 mm) for imaging and staining. The microspheres delineate the non-perfused area (AAR). The infarct size (I) was determined by staining in 1% 2,3,5 triphenyl tetrazolium chloride (TTC) followed by 10% formalin bath overnight .

Experimental design:



RESULTS

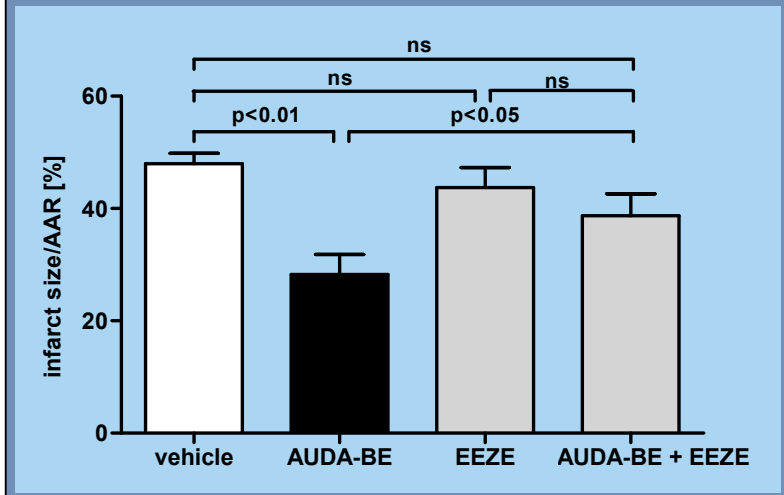


Figure 1:Effect of AUDA-BE on myocardial infarct size
Myocardial infarct size data expressed as a percent of the area at risk (I/AAR) in the 4 groups. Intraperitoneal injection of AUDA-BE prior to reperfusion reduced I/AAR. This protection was abolished by pre-treatment with the EET antagonist 14,15-EEZE (means±SEM, n=5 per group).

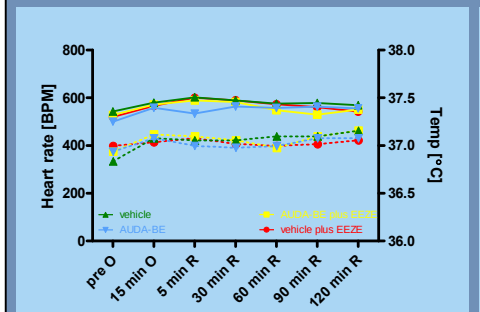


Figure 2: Heart rate and rectal temperature.
Heart rate variability shows no difference between the 4 groups tested. Temperature was tightly controlled throughout the experiments at a target rectal temperature of 37 ºC and was similar between groups. Solid line indicates heart rate and dashed line rectal temperature (2-way-ANOVA, n=5 per group)

	Body Weight (g)	Bi-ventricular Weight (mg)	Area At Risk (mm³)	Infarct Volume (mm³)	AAR/Bi-ventricular Volume (%)
vehicle	27±0	121±3	40±3	19±1	30±2
AUDA-BE	26±1	120±8	46±4	14±3	35±1
EEZE	27±1	122±4	42±4	18±2	33±4
EEZE+ AUDA-BE	27±1	122±9	42±5	16±3	32±4

Figure 3: *Weight and* Volumes
Total infarct volume was smaller and AAR volume and AAR/biventricular volume was larger in animals treated with AUDA-BE compared to the other groups. Body weight and biventricular heart size are similar in all groups tested (mean ± SEM, n=5 per group)

CONCLUSION

- Inhibition of sEH prior to reperfusion elicits cardioprotection in vivo via 14,15-EET mediated pathway.
- Pharmacological inhibition of sEH may serve as a novel therapeutic option for cardioprotection against myocardial ischemia-reperfusion injury in the perioperative setting.

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