14,15-Epoxyeicosatrienoic Acid Elicits Pre- and Post-Conditioning Protection Against Myocardial Ischemia-Reperfusion Injury in Mice

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ABSTRACT – #1024

14,15-epoxyeicosatrienic acid (14,15-EET) is a polyunsaturated fatty acid derived from arachidonic acid. It is released during ischemia and rapidly broken down via soluble epoxide hydrolase (sEH). sEH knockout mice have increased ischemic tolerance vs. wild-type. We investigated whether exogenous 14,15-EET elicits cardioprotection when given prior to occlusion (pre-conditioning) or at reperfusion (post-conditioning). We subjected male C57BL\6J mice to 40 min of left coronary artery occlusion and 2 h reperfusion. 14,15-EET or vehicle (25% ethanol) was administered 15 min before occlusion (pre-conditioning group), or 5 min before reperfusion (post-conditioning group). Area-at-risk (AAR) and infarct size (I) were assessed using fluorescent microspheres and triphenyl tetrazolium chloride. Infarct size is expressed as I/AAR (mean±SEM). I/AAR was reduced in the EET pre-conditioning group (24.2±3% n=5 vs. 54.1±3% n=6, p<0.001), and in the post-conditioning group (35.8±1% n=5 vs. 47.7±1% n=5, p<0.01), as compared to vehicle. In summary, 14,15-EET has both pre-conditioning and post-conditioning effects in mouse hearts.

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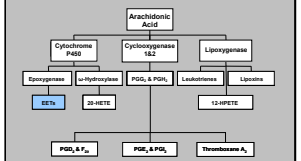
CONCEPT

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 terminated by hydration into less active dihydroeicosatrienoic acids (DHETs) by soluble epoxide hydrolase (sEH). Functional recovery is improved in isolated hearts from sEHKO mice after global ischemia (5).

HYPOTHESIS

Pre-ischemic and Post-ischemic administration of 14,15-EET reduces infarct size/area at risk (I/AAR) after regional myocardial ischemia-reperfusion in vivo.

METHOD

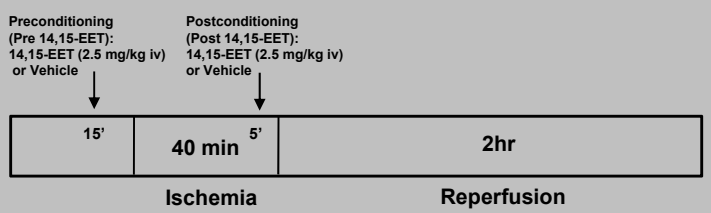
Animals:

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:



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4) Gross et al.J Mol Cell Cardiol (2007) 42: 687-691;
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RESULTS

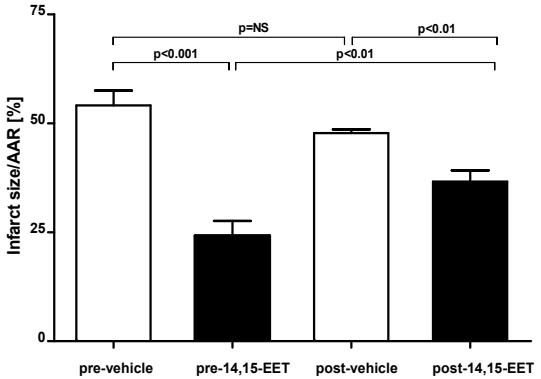

Figure 1:Effect of 14,15-EET on myocardial infarct size
Myocardial infarct size data expressed as a percent of the area at risk (I/AAR) in the 4 groups. I/AAR was reduced in the 14,15-EET pre-conditioning group, and also in the 14,15-EET post-conditioning group, as compared to their respective vehicle controls (means±SEM, n=5 -6 per group).

	Preconditioning vehicle		Preconditioning 14,15-EET		Postconditioning vehicle		Postconditioning 14,15-EET	
Body weight, g	28±0.6		27±0.4		28±0.3		27±1.0	
Biventricular weight, mg	131±3		131±4		128±3		127±5	
	HR	T	HR	T	HR	T	HR	T
Pre-occlusion	581±12	37.1±0.04	595±10	37.0±0.07	514±33	36.9±0.09	548±22	36.8±0.07
15min after occlusion	593±18	37.0±0.03	614±11	37.0±0.03	576±20	36.9±0.02	542±41	37.0±0.05
5min after reperfusion	589±16	37.0±0.02	586±9	36.9±0.03	588±10	37.0±0.04	597±18	37.1±0.05
30min after reperfusion	575±16	37.0±0.05	563±23	37.0±0.03	581±16	37.0±0.04	570±21	37.0±0.05
60min after reperfusion	543±14	37.0±0.05	543±29	36.8±0.02	571±18	37.04±0.05	541±24	36.9±0.02
90min after reperfusion	549±11	37.0±0.05	539±23	37.0±0.02	555±23	37.0±0.06	525±20	37.0±0.02
120min after reperfusion	556±8	37.0±0.05	575±9	37.04±0.05	555±19	37.0±0.04	536±17	37.0±0.05

Figure 4: Physiological parameters
Heart rate and temperature at all time points between 4 groups were not significantly different. (mean ± SEM)

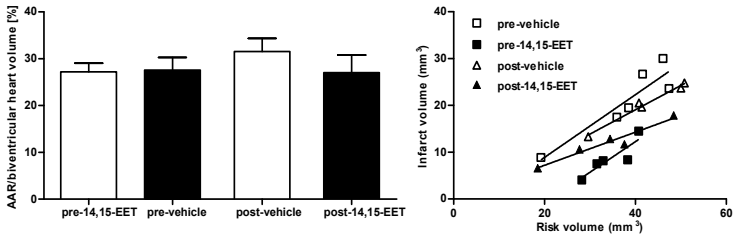

Figure 2: (A) AAR/biventricular volume in the 4 groups. There were no significant differences between groups. (B) Linear regression of risk volume/infarct volume in the 4 groups. Linear regression of infarct volume to risk volume showed no significant difference in slope but a significantly different y-axis intercept between the groups (14,15-EET pre-conditioning vs. pre-vehicle control, p<0.001; and 14,15-EET post-conditioning vs. post-vehicle control, p<0.01).

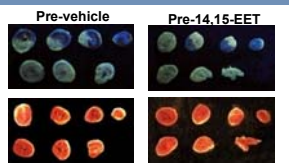

Figure 3A: Images of hearts (pre-vehicle vs. pre-14,15-EET)
Top row: fluorescent images for AAR analysis
Bottom row: TTC staining for infarct size analysis

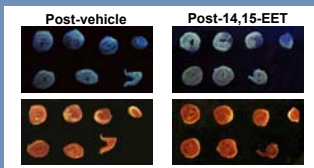

Figure 3B: Images of hearts (post-vehicle vs. post-14,15-EET)
Top row: fluorescent images for AAR analysis
Bottom row: TTC staining for infarct size analysis

CONCLUSION

- 14,15-EET prior to ischemia (preconditioning) and at reperfusion (postconditioning) reduces I/AAR in vivo after regional myocardial ischemia-reperfusion.
- 14,15-EET preconditioning is more effective than postconditioning in I/AAR reduction.