Soluble Epoxide Hydrolase Inhibition Attenuates Cardiac Remodelling Post-Myocardial Infarction

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Introduction
Exposure of cardiomyocytes to hypertension, diabetes, and chronic heart failure results in pathological myocardial remodelling, an adaptive process aimed at preserving cardiac function. TGFβ and CTGF are profibrotic and hypertrophic mediators, respectively, and their expression is increased in response to abnormalities in the cardiac milieu. The soluble epoxide hydrolase (sEH) is an enzyme ubiquitously expressed in multiple tissues, including the heart, that catalyzes the metabolism of epoxyeicosatrienoic acids (EETs) to dihydroxyeicosatetraenoic acids (DHETs). EETs exert protective effects on the cardiovascular system. The sEH inhibitor GSK2188931 (GSK) has been shown to reduce cardiac hypertrophy and fibrosis in animal models of myocardial infarction (MI) and ischaemia-reperfusion. Here, we investigated the effects of GSK treatment on cardiac remodelling, with a focus on hypertrophy and fibrosis, and assessed the impact of sEH inhibition on sEH activity, collagen expression, and the expression of profibrotic mediators TGFβ and CTGF in vitro and in vivo.

Methods

Cell Culture: Cardiac myocytes and fibroblasts were isolated from 1-2 day old rat hearts by collagenase digestion. Experimental Design: Cells were serum starved for 48h prior to addition of the sEH inhibitor GSK2188931 (GSK: 10⁻⁷, 3×10⁻⁸M) and stimulation 4h later. Cardiac myocytes were stimulated with angiotensin II (AngII, 10⁻⁷M) for 6h together with H-Leucine. Hypertrophy was assessed by H-Leucine incorporation. Cardiac fibroblasts were stimulated with transforming growth factor β1 (TGFβ1, 10ng/ml) together with H-Proline for 4h. Collagen synthesis was assessed by H-Proline incorporation. Gene Studies: The above experiments were repeated with 24h stimulation. Tissues were harvested and processed for histology and gene expression. Statistics: Data are expressed as mean ± SEM. All data were analysed by one way ANOVA. * P<0.05, ** P<0.01, *** P<0.001 compared to Unstimulated or Sham-operated groups.

Results

In Vivo: Myocardial infarction (MI) was induced in male Sprague-Dawley rats by LAD ligation. Immediately post-surgery animals were randomised into groups receiving either the sEH inhibitor GSK2188931 (1000ppm in food) or standard rat chow as the vehicle. Five weeks after treatment, cardiac function was assessed by 2-D and M-mode echocardiography, and cardiac catherisation with Millar pressure-volume loop analysis. Tissues were harvested and processed for histology and gene expression studies.

Acknowledgements
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References:

Figure 1: In cardiac myocytes, the sEH inhibitor, GSK, dose-dependently reduced AngII-stimulated hypertrophy as well as ANP and β-MHC gene expression.

Figure 2: In cardiac fibroblasts, the sEH inhibitor, GSK, reduced TGFβ-stimulated collagen synthesis at 3µM, and reduced TGFβ, CTGF and α(sm)pro-collagen I gene expression at all doses of GSK.

Figure 3: Representative picrosirius red stained images of the non-infarct zone from the LV. GSK reduced interstitial picrosirius red staining in the non-infarct and peri-infarct zones of the LV compared to vehicle-treated animals.

Figure 4: GSK reduced collagen I immunohistochemistry in the non-infarct and peri-infarct zones of the LV compared to vehicle-treated animals.

Figure 5: GSK indicated a trend toward reduced myocyte cross sectional area (MCSA) compared to vehicle-treated animals.

Figure 6: Inflammatory cells: GSK reduced interstitial macrophage infiltration in the peri-infarct zone of the LV compared to vehicle-treated animals.

Figure 7: Echocardiography: GSK attenuated the reduction LV Ejection Fraction and Fractional Shortening in vehicle-treated animals.

Figure 8: Haemodynamics and Millar analysis: GSK showed trends toward improving LV end diastolic pressure, contractility (dp/dtmax), and end systolic pressure-volume relationship compared to vehicle-treated animals.

Summary
In vitro, soluble epoxide hydrolase inhibition reduced myocyte hypertrophy and cardiac fibroblast collagen synthesis including expression of implicated genes. Post-MI, sEH inhibition attenuated interstitial cardiac fibrosis, hypertrophy and macrophage infiltration whilst improving some measures of cardiac function.

Conclusion
Soluble epoxide hydrolase inhibition has beneficial anti-remodelling effects in a post-MI setting. The actions of soluble epoxide hydrolase inhibition may provide a novel avenue for pharmacological therapy in the treatment of post-MI cardiac remodelling.

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