Sunitinib mediates mitochondrial ROS production in adult rat cardiac fibroblasts via CaMKII oxidation

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Abstract

Introduction Ca^{2+}/calmodulin dependent protein kinase II (CaMKII) is a central mediator of Ca^{2+}-induced signalling in the heart and regulates both normal cardiac physiology and pathology. Sunitinib malate is an oral Type I tyrosine kinase inhibitor (TKI) known to inhibit more than 50 kinases, with anti-angiogenic and anti-proliferative effects affiliated with off-target cardiotoxicity. Previous work has shown that chronic sunitinib treatment significantly increases CaMKII expression and activity and this correlates with significant cardiac dysfunction in vivo.[1] Mitochondrial dysfunction, mediated by increased mitochondrial Ca^{2+} and resultant mitochondrial ROS production, has been proposed as an underlying mechanism for TKI-induced cardiotoxicity in cardiomyocytes.[2] However, little is known of how TKIs may affect the non-contractile cells of the heart. Here, we have investigated whether sunitinib treatment increases mitochondrial ROS production in cardiac fibroblasts (CF) and whether CaMKII may play a role in this potential cardiotoxic mechanism.

Methods CF were isolated from adult Sprague-Dawley rats (male, 250–350 g, n=8) via bulk collagenase digestion and were maintained in culture before treatment with sunitinib (Cayman Chemical Company, Michigan, USA) (1–10µM) for 16 hours in the presence of serum (DMEM supplemented with 20% FBS). Mitochondrial superoxide production was assessed using MitoSOX Red (ThermoFisher Scientific, UK). CF were pre-treated with 5µM KN-93 (2 hours) Sigma-Aldrich, UK) to inhibit CaMKII before the addition of 10µM sunitinib for 16 hours. MitoSOX Red analysis was then repeated as before. Data are presented as mean values ± S.E.M of *n* observations, where *n* represents the number of samples. Comparisons were assessed by one-way ANOVA with post hoc Dunnett's test.

Results MitoSOX fluorescence imaging revealed a significant increase in mitochondrial superoxide production in sunitinib treated CF (144.6±1.1 vs 526.2±24.0 vs 178.8±4.6 relative fluorescent units; control vs 10µM sunitinib vs 10µM antimycin A, n=3, $p<0.05$). However, KN-93 pre-treatment significantly reduced mitochondrial ROS production in sunitinib treated CF (440.2±0.6 vs 680.5±5.6 vs 576.1±11.4 relative fluorescent units; control vs 10µM sunitinib vs 10µM sunitinib + 5µM KN-93, n=3, $p<0.05$).

Conclusion Sunitinib treatment increases mitochondrial superoxide production in CF, supporting a previously proposed TKI-induced cardiotoxic mechanism mediated by mitochondrial dysfunction. This cardiotoxic mechanism may be mediated by CaMKII (activated via oxidation), although further work is required to corroborate this. Future work will determine the effect this mechanism has on mitochondrial function and establish whether targeted CaMKII inhibition can reduce/reverse TKI-induced cardiotoxic effects.

References

1. Mooney, L., et al. *British Journal of Pharmacology*. 2015;172:4342–4354.
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