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PRECLINICAL RESEARCH

Increased Afterload Augments Sunitinib-Induced Cardiotoxicity in an Engineered Cardiac Microtissue Model

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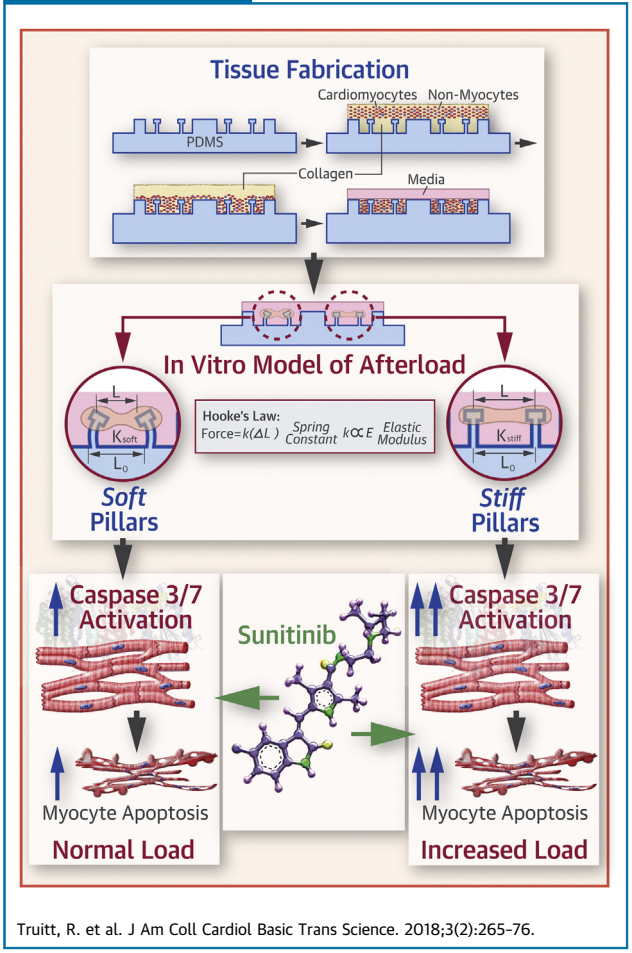
VISUAL ABSTRACT



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HIGHLIGHTS

- Sunitinib, an oral tyrosine kinase inhibitor used widely to treat solid organ tumors, frequently induces hypertension and causes LV dysfunction in up to 19% of treated individuals.
- Sunitinib-induced cardiotoxicity can be modeled using engineered CMT.
- In CMT, sunitinib induces dose- and duration-dependent activation of apoptosis pathways and decreases in CMT force generation, spontaneous beating, and mitochondrial membrane potential.
- Exposure of CMT to increased in vitro afterload intensifies the cardiotoxicity of clinically relevant sunitinib concentrations.
- These findings suggest that intensive antihypertensive therapy may be an appropriate strategy to mitigate LV dysfunction observed in patients treated with sunitinib.

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ABBREVIATIONS AND ACRONYMS

2D = 2-dimensional

3D = 3-dimensional

AICAR = 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside

AMPK = adenosine monophosphate-activated protein kinase

ATP = adenosine triphosphate

CCCP = carbonyl cyanide *m*-chlorophenyl hydrazine

CMT = cardiac microtissue

DMSO = dimethyl sulfoxide

EDTA = ethylenediamine tetraacetic acid

huMSC = human mesenchymal stem cell

Hu-iPS-CM = human induced pluripotent stem cell cardiomyocyte

iPS-CM = induced pluripotent stem cell-derived cardiomyocyte

LV = left ventricle

NRVM = neonatal rat ventricular myocyte

PDMS = polydimethylsiloxane

RPMI = Roswell Park Memorial Institute medium

TMRM = tetramethylrhodamine

SUMMARY

Sunitinib, a multitargeted oral tyrosine kinase inhibitor, used widely to treat solid tumors, results in hypertension in up to 47% and left ventricular dysfunction in up to 19% of treated individuals. The relative contribution of afterload toward inducing cardiac dysfunction with sunitinib treatment remains unknown. We created a preclinical model of sunitinib cardiotoxicity using engineered microtissues that exhibited cardiomyocyte death, decreases in force generation, and spontaneous beating at clinically relevant doses. Simulated increases in afterload augmented sunitinib cardiotoxicity in both rat and human microtissues, which suggest that antihypertensive therapy may be a strategy to prevent left ventricular dysfunction in patients treated with sunitinib. (J Am Coll Cardiol Basic Trans Science 2018;3:265–76)

The rise of small molecule inhibitors targeting receptor tyrosine kinases that regulate tumor vasculature angiogenesis and cellular proliferation have resulted in important gains in cancer survival (1). However, many of these "targeted" therapies have unintended consequences on the cardiovascular system (2–5). Sunitinib, a multitargeted tyrosine kinase inhibitor used widely in the treatment of renal cell carcinoma, gastrointestinal stromal tumors, and neuroendocrine tumors, is currently under investigation in over 500 active clinical trials (6–8). However, among sunitinib-treated patients, hypertension occurs in 11% to 43% of patients and left ventricular (LV) dysfunction in up to 19% (9–11). These toxicities, although often manageable, can result in dose reduction or treatment interruption, which can affect oncologic outcomes.

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Cardiovascular toxicity with sunitinib has been hypothesized to be a result of off-target inhibition of receptor tyrosine kinases and mitochondrial function that are important for maintaining cardiovascular homeostasis (3,6,12–14), particularly during states of increased stress (15–19). However, the relative contribution of each of these factors remains poorly understood. Another contributing factor that may be critical in the development of LV dysfunction during sunitinib treatment is hypertension (20–22). More specifically, it is not clear whether hypertension unmasks LV dysfunction or actually lowers the threshold for sunitinib cardiotoxicity. We hypothesized that increased afterload augments the cardiotoxic effects of sunitinib.

Testing this hypothesis in humans would likely require substantial resources and involve ethical challenges with cohorts of patients with untreated hypertension. Current in vitro cell culture and animal models also suffer from limitations that minimize their usefulness for modeling how biomechanical influences affect sunitinib cardiotoxicity in humans (23,24). Thus, we used an engineered in vitro 3-dimensional (3D) cardiac microtissue (CMT) model incorporating cardiomyocytes from neonatal rats or human pluripotent stem cells that self-assemble onto polydimethylsiloxane (PDMS) pillars (25,26). We used this system to characterize sunitinib cardiotoxicity using metrics for cell viability, mitochondrial dysfunction, and cardiac function, and examined how these characteristics are affected by sunitinib dose, treatment duration, and the magnitude of biomechanical loading.

METHODS

CMT PLATFORM. CMT arrays were fabricated as previously described (25,26) (Supplemental Appendix). Devices were cast from PDMS pre-polymer (5:1 to 15:1

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