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PRE-CLINICAL RESEARCH

Chemotherapeutic Efficacy of Phosphodiesterase Inhibitors in Chagasic Cardiomyopathy

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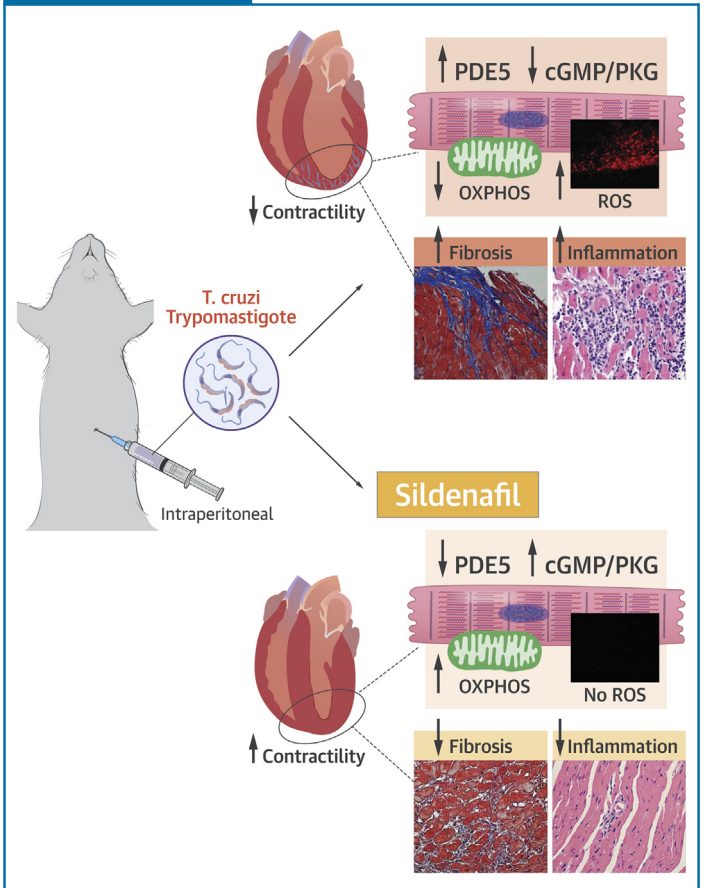
VISUAL ABSTRACT



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HIGHLIGHTS

- Mice infected with *T. cruzi* control acute parasitemia but develop chronic chagasic cardiomyopathy.
- Treatment with SIL (a phosphodiesterase inhibitor) during a therapeutic window of indeterminate phase provided powerful cardioprotective effects against chronic development of cardiomyopathy and LV dysfunction.
- SIL normalized the cGMP-dependent protein kinase activity and mitochondrial oxidative metabolism, and established the oxidant/antioxidant balance in chagasic myocardium.
- SIL prevented the oxidative/inflammatory adducts that precipitate cardiomyocytes death and cardiac remodeling in CCM.

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SUMMARY

Molecular mechanisms of *Trypanosoma cruzi* (*Tc*)-induced Chagasic cardiomyopathy (CCM) are not well understood. The NO-cGMP-PKG1α pathway maintains cardiac homeostasis and inotropy and may be disturbed due to phosphodiesterase (PDE5)-mediated cGMP catabolism in CCM. To test this, C57BL/6 mice were infected with *T. cruzi*, and after the control of acute parasitemia (~45 days post-infection), given sildenafil (SIL) (1 mg/kg) treatment for 3 weeks that ended long before the chronic disease phase (~150 days post-infection). The PDE5 was increased and cGMP/PKG activity was decreased in chagasic myocardium. Transthoracic echocardiography revealed left ventricular (LV) systolic function, that is, stroke volume, cardiac output, and ejection fraction, was significantly decreased in chagasic mice. SIL treatment resulted in normal levels of PDE5 and cGMP/PKG activity and preserved the LV function. The cardioprotective effects of SIL were provided through inhibition of cardiac collagenosis and chronic inflammation that otherwise were pronounced in CCM. Further, SIL treatment restored the mitochondrial DNA–encoded gene expression, complex I–dependent (but not complex II–dependent) ADP-coupled respiration, and oxidant/antioxidant balance in chagasic myocardium. In vitro studies in cardiomyocytes verified that SIL conserved the redox metabolic state and cellular health via maintaining the antioxidant status that otherwise was compromised in response to *T. cruzi* infection. We conclude that SIL therapy was useful in controlling the LV dysfunction and chronic pathology in CCM. (J Am Coll Cardiol Basic Trans Science 2016;1:235–50)

ABBREVIATIONS AND ACRONYMS

ANOVA = analysis of variance
CCM = chagasic cardiomyopathy
cGMP = cyclic guanosine monophosphate
C_t = threshold cycle
DCF = dichlorodihydrofluorescein
DHE = dihydroethidium
DMSO = dimethyl sulfoxide
EF = ejection fraction
ER = endoplasmic reticulum
GC = guanylyl cyclase
HRP = horseradish peroxidase
K-W = Kruskal-Wallis
LDH = lactate dehydrogenase
LPO = lipid hydroperoxide
LV = left ventricle/ventricular
mtDNA = mitochondrial DNA
NO = nitric oxide
OD = optical density
PDE = phosphodiesterase
pi = post-infection
PKG = cGMP-dependent protein kinase
QR1 = quartile 1
RCR = respiratory control ratio
ROS = reactive oxygen species
SIL = sildenafil
SV = stroke volume
Tc = *Trypanosoma cruzi*

Chagasic cardiomyopathy (CCM) is an illness initiated by *Trypanosoma cruzi* infection. In Latin America, ~13 million people are infected and 120 million are believed to be at risk of infection via vectorial, congenital, and other modes of transmission (1). Vectorial transmission occurs in the United States (2), and >300,000 infected individuals living in the United States can potentially transfer infection through blood and organ donation (3–5). Approximately, 30% to 40% of the infected individuals manifest prolonged micro and macro cardiac injuries that cause hypertrophy and increased stiffness of the left ventricular (LV) walls, and ultimately lead to a clinical presentation of ventricle arrhythmia, thromboembolism, and heart failure (6). Due to a lack of accurate therapies (7), CCM causes approximately $8 billion/year in costs in health care and loss of productivity (8).

Molecular mechanisms of *T. cruzi* (*Tc*)-induced CCM are not well understood. Early upon infection, *T. cruzi* is shown to upregulate in human cardiac myocytes the expression of several transcription factors and cytokines/chemokines implicated in the development of fibrogenic response (9). Chronic progression of CCM is associated with persistent increase in circulatory and myocardial inflammatory and oxidative stress in human patients and experimental models (reviewed in [10]). Although inflammatory stress is believed to be present in response to *T. cruzi* infection, we showed that mitochondrial inefficiency of respiratory chain was the major source of reactive oxygen species (ROS) in the heart (11). Treatment of chagasic mice with a ROS scavenger (12) and mice genetically enhanced in their capacity to scavenge mitochondrial ROS (13) were better equipped in handling the oxidative and inflammatory stress, thus suggesting that ROS may signal chronic inflammation during CCM.

In the heart, nitric oxide (NO) and atrial natriuretic peptide signal the activation of guanylyl cyclase (GC) that produces cyclic guanosine monophosphate (cGMP) (14). The cGMP binding activates cGMP-dependent protein kinase (PKG). PKG phosphorylates serine and threonine residues on many cellular proteins and mediates the downstream effects in maintaining the force of contraction of cardiac myocytes (15) through regulating cytosolic free Ca^{2+} level and sensitivity of muscle fibers to Ca^{2+} (16). Others have implied that cGMP/PKG regulate the activities of phosphodiesterases (PDEs) that hydrolyze cyclic nucleotides (17). Of the 4 PDEs that are expressed in the heart and, in a feedback mechanism, that hydrolyze cyclic nucleotides, PDE5 is the only known cardiac phosphodiesterase that selectively hydrolyzes cGMP and negatively regulates cardiac inotropy (18). An observation of PDE5 subcellular localization to myocyte z-bands (19) implies that it may exert its effects on cGMP/PKG signaling in a spatiotemporal manner and determine the functional outcomes in stressed heart. The NO-cGMP-PKG signaling may also occur in mitochondria and play a role in ischemic preconditioning and antioxidant cardioprotection (20), though the exact mechanism remains to be identified.