Chronic Cyclosporine Treatment Does Not Reduce Total LV Collagen and Fibrosis in Mini-Swine with Heart Failure

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ABSTRACT

Cardiac extracellular matrix remodeling is a pathological process that may negatively affect the mechanical properties of the heart in patients with heart failure with preserved ejection fraction (HFpEF). The remodeling process is partially regulated by the loss of cardiomyocytes through cell death pathways mediated in part by the mitochondria. Our laboratory previously showed low intensity interval exercise training attenuates mitochondrial dysfunction, characterized by increased mitochondrial permeability transition (MPT). Conventional treatments have failed to improve the prognosis of HFpEF patients, and there is a critical need for generating novel treatment options for those diagnosed with the disease. Therefore we hypothesized a reduced, non-immunosuppressive dose of the drug cyclosporine (CsA; a general cyclophilin inhibitor) would block MPT via inhibition of cyclophilin D, a key component of the MPT pore, and attenuate the development of HFpEF via inhibition of cell death pathways and subsequent fibrotic myocardial remodeling. The purpose of this study was to examine the effects of CsA on extracellular matrix remodeling in aortic-banded mini-swine divided into three groups (n=5); control non-banded (CON), HFpEF non-treated (HF), and HFpEF treated with CsA (HF-CsA; 2 mg/kg/day). CsA treatment began 6 weeks after banding and continued for 14 weeks. Tissue was isolated from the left ventricle (LV). Picrosirius Red Stain was used to determine total LV collagen and Masson's Trichrome Stain was used to determine total LV fibrosis. Fibrotic remodelling was assessed as percent area and density. The percent area of both collagen and fibrosis increased by approximately 35% in both aortic banded groups regardless of treatment. Collagen staining density was increased only in the HF group. In conclusion, CsA treatment did not decrease total LV collagen or fibrosis in heart failure. Future directions include examination of regulators of fibrotic remodeling, including Matrix Metalloproteinases (MMPs) and their Tissue Inhibitors (TIMPs).

Objective

The objective of this study was to examine the effects of CsA on LV extracellular matrix remodeling in a Yucatan miniature swine model of HEpEF.

Hypothesis

CsA treatment will reduce total collagen and fibrosis in aortic-banded Yucatan miniature swine.

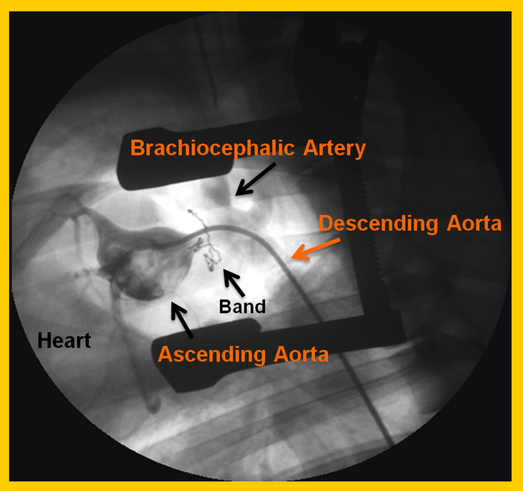
METHODS

Cyclosporine Treatment
In the presence of existing LV hypertrophy (six weeks post-surgery), animals began Cyclosporine treatment. Animals were dosed 2mg/kg/day for a duration of 14 weeks.

Groups:
- Control non-banded (CON); n=5
- HFpEF non-treated (HF); n=5
- HFpEF treated with CsA (HF-CsA); n=5

Aortic Banding Procedure: LV hypertrophy/HF was induced by aortic banding. The aortic band was placed on the ascending aorta proximal to the brachiocephalic artery. A systolic trans-stenotic gradient of ≈ 70 mmHg (73 ± 2 & 74 ± 1 mmHg for HF & HF-CsA, respectively, P = nonsignificant [NS]) was achieved while maintaining a distal peripheral vascular MAP of 90 mmHg (93 ± 1 & 90 ± 1 mmHg for HF and HF-CsA, respectively, P = NS) under anesthesia using phenylephrine (1–3 $g \cdot kg^{-1} \cdot min^{-1}$ iv) at a heart rate (HR) of 100 beats/min (100 ± 5 and 107 ± 2 beats/min for HF and HF-CsA, respectively, P = NS).

Histology and immunohistochemistry. Cross-sections of LV were formalin fixed, embedded in paraffin, and immunohistochemistry stained for the assessment of fibrosis and collagen. Briefly, total fibrosis was visualized from 4-m-thick sections of the LV using Masson's trichrome stain, and total collagen was visualized using Picrosirius red staining with previously established methods. Fibrosis and collagen were quantified from 4 separate fields/animal using Image-Pro Plus analysis software (version 6.2, MediaCybernetics, Bethesda, MD) and expressed as the percent area stained and density of the stain.



RESULTS

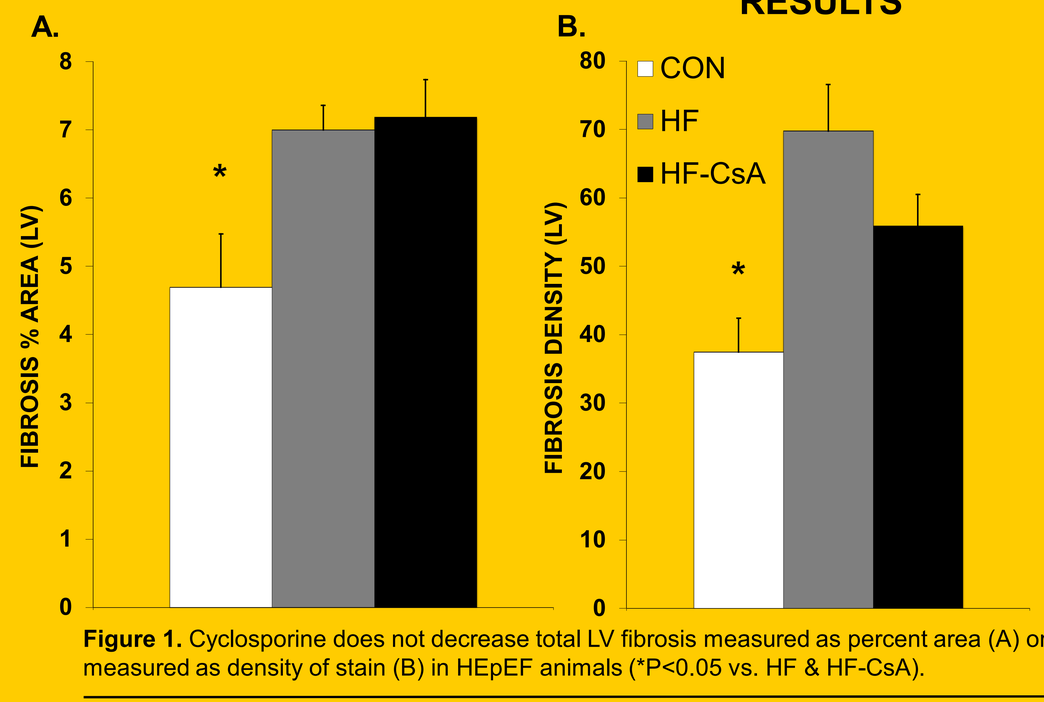


Figure 1. Cyclosporine does not decrease total LV fibrosis measured as percent area (A) or measured as density of stain (B) in HEpEF animals (*P<0.05 vs. HF & HF-CsA).

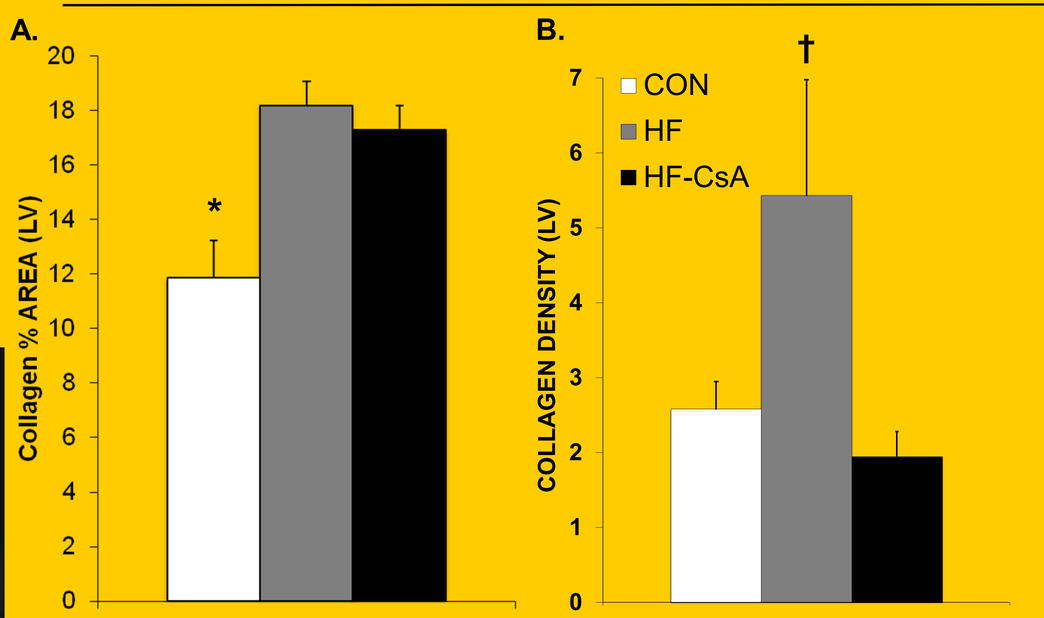
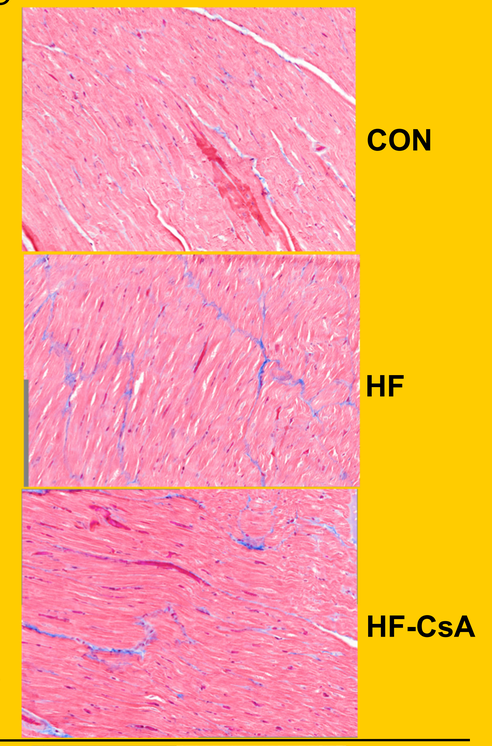
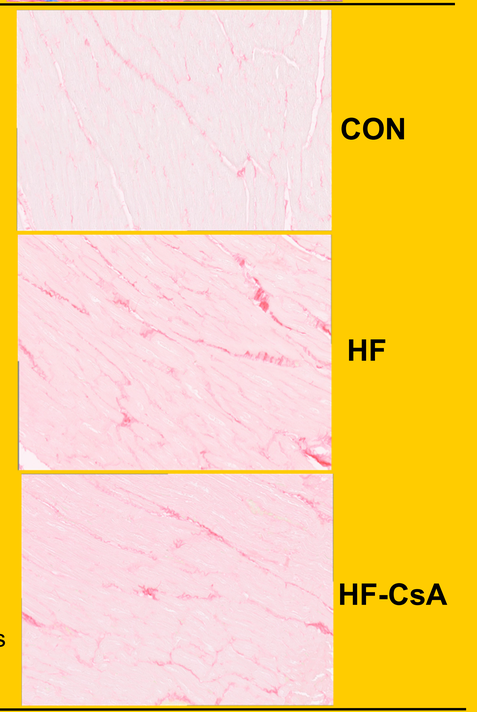


Figure 2. A, CsA does not decrease total LV collagen measured as percent area. B, CsA does decrease LV collagen density. (*P<0.05 vs. HF & HF-CsA; †P<0.05 vs. CON & HF-CsA).





CONCLUSION

Chronic cyclosporine treatment does not decrease total LV collagen or fibrosis in a mini-swine model of HEpEF. Our results suggest cyclosporine is not a viable therapeutic treatment for HF.

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