

# Humoral factors in serum of rats with chronic heart failure induce cardiomyocyte hypertrophy and reduce viability

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## Introduction

Myocardial infarction (MI) is one of the leading causes of global mortality. Over the last decade, acute MI mortality has significantly decreased; however, fatal heart failure remains a common complication for post-MI survivors.<sup>1</sup> Lack of oxygen due to MI causes cardiac tissue necrosis and, as a result, the induction of a pro-inflammatory environment. The necrotic tissue is replaced with a fibrotic scar rather than new cardiomyocytes. Excessive inflammation can result in increased death of cardiomyocytes and increased fibrosis, which reduces cardiac function.<sup>2</sup> To optimise cardiac function, the heart may undergo hypertrophy. Although initially the hypertrophic response is advantageous, sustained hypertrophy is a contributor to heart failure.<sup>3</sup>

Here, we assess whether rodent chronic heart failure (CHF) serum contributes to the induction of cardiomyocyte hypertrophy and cell death.

## Materials and methods

MI was induced by the surgical ligation of the left anterior descending (LAD) artery in adult male Sprague Dawley rats. Serum was collected from healthy controls, and 2 weeks (acute) and 16 weeks (CHF) post-MI.

Primary adult cardiomyocytes were isolated from healthy rats and treated with control and CHF serum for 24 and 48 hours for apoptosis and cytotoxicity assays.

Serum from healthy and CHF rats was further used to stimulate the HL-1.6 cardiomyocyte cell line for quantitative PCR (qPCR) expression analysis of  $\alpha$ -myosin heavy chain (*MYH6*) gene, and the hypertrophic genes  $\beta$ -myosin heavy chain (*MYH7*), atrial natriuretic factor (*NPPA*) and brain natriuretic peptide (*NPPB*).

## Results and discussion

CHF serum applied to healthy adult cardiomyocytes for 24 h resulted in increased apoptosis compared to stimulation with healthy serum ( $25.91 \pm 4.53$  (mean  $\pm$  standard error of the mean; control;  $n=5$ );  $53.13 \pm 3.961$  (CHF;  $n=10$ );  $p=0.0025$ ). CHF

serum stimulation for 24 h and 48 h showed reduced viability due to increased cytotoxicity compared to stimulation with healthy serum (day 1:  $83.75 \pm 0.9287$  (control;  $n=5$ );  $73.45 \pm 2.722$  (CHF;  $n=7$ );  $p=0.0178$ ; day 2:  $86.66 \pm 1.294$  (control;  $n=5$ );  $73.43 \pm 3.508$  (CHF;  $n=7$ ),  $p=0.0066$ ).

The increase in apoptosis and cell death of cardiomyocytes after CHF serum stimulation suggests the presence of humoral factors in CHF serum that cause direct damage to the cardiomyocytes.

To assess the effect of CHF on cardiac hypertrophy, CHF serum was applied to a cardiomyocyte cell line (HL-1.6). This did not cause a significant change in the expression of *NPPA*, *NPPB* and *MYH6* compared to control serum. However, CHF serum induced a significant increase in *MYH7* expression compared to control (fold change  $0.88 \pm 0.08$  (control;  $n=5$ );  $1.2 \pm 0.05$  (CHF;  $n=8$ );  $p=0.0061$ ). Importantly, the *MYH7/MYH6* ratio also increased (fold change  $2.59 \pm 0.58$  (control;  $n=5$ );  $4.19 \pm 0.49$  (CHF;  $n=8$ );  $p=0.0322$ ).

A previous study suggests that the increase in *MYH7* alone is sufficient to cause cardiac deterioration.<sup>4</sup> Furthermore, it has been proposed that the shift from *MYH6* to *MYH7* expression might play a role in cardiac disease progression.<sup>4</sup> Thus, the increase in *MYH7* expression and *MYH7/MYH6* ratio indicates a hypertrophic response.

## Conclusion

These findings suggest that humoral factors present in CHF serum may be involved in the induction of the cellular processes leading to persistent myocardial damage and pathological cardiac hypertrophy. ■

## Conflicts of interest

None declared.

## References

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