Death Receptor 5 provides protection in a hypertension-induced model of heart failure

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Abstract

Cardiovascular disease is one of the world's leading health problems. In particular, death of cardiomyocytes as a result of chronic stress or injury results in pathological remodeling leading to heart failure. Therapies preventing cardiomyocyte death and maladaptive remodeling decrease the decline in cardiac function and improve health. Death Receptor (DR) 5 has been extensively analyzed for its role in cancer, but significantly less regarding cardiac function. Studies have shown that DR5 activates non-canonical mechanisms in cardiomyocytes and may provide cardio protection against cardiomyocyte death and cardiac fibrosis. Thus, it was hypothesized that mice that lack DR5 will have an exasperated cardiac phenotype in response to chronic pressure-overload. To address this question, wild-type (WT) C57BL/6 or global DR5 knockout (KO) mice were administered phenylephrine and angiotensin II for four weeks via osmotic minipump to induce hypertension Blood pressure was monitored over time by conscious, noninvasive blood pressure monitoring using the Kent CODA monitoring system. and cardiac parameters were examined by serial echocardiography. At the termination of the study, cardiac hypertrophy was assessed by gravimetric analysis, wheat germ agglutinin staining to determine cardiomyocyte size and induction of the fetal gene program. Fibrosis was quantified by Masson's trichrome staining, picrosirius red staining and collagen expression. It was observed that the DR5KO has a lower susceptibility to heart failure. The administration of phenylephrine and angiotensin II caused significantly less hypertrophy of the individual cardiomyocytes, and the heart overall compared to the wildtype mice. These results would suggest that DR5 activation invoked a hypertensive state in the heart and increase expression of pro hypertrophic genes.

Introduction

- Proceeding studies have looked at the causative agents responsible for apoptosis of cardiomyocytes.
- One mechanism that has been explored more recently regarding heart failure is TRAIL ligand and its receptor, Death Receptor 5. DR5 has shown to activate mechanisms associated with cardio protection instead of cardiomyocyte death and fibrosis .
- In the past, there have been many different studies looking at the effects of TRAIL and Death Receptor 5 regarding cancer treatments, but none specifically targeting their effect elsewhere in the body – specifically the heart.
- There is suggestion that DR5 activation is potentially antifibrotic.

Methods

Figure 1. Day 0- Baseline data is recorded using the Kent Scientific Coda monitoring system to measure blood pressure and VEVO 2100 for echocardiography. After this data is collected, osmotic pumps containing phenylephrine and angiotensin II are implanted into WT and DR5KO mice. Day 14- Data is recorded again on day 14 using the the Kent Scientific Coda monitoring system to measure blood pressure and VEVO 2100 for echocardiography. After this data is collected, the original osmotic pumps containing phenylephrine and angiotensin II are replaced with new pumps in WT and DR5KO mice. Day 28- Final data is recorded on day 28 using the the Kent Scientific Coda monitoring system to measure blood pressure and VEVO 2100 for echocardiography. After this data is collected, the mice are sacrificed. The heart is harvested from each specimen for histology and PCR testing. Histology is preformed utilizing picrosirius red staining, Masson's Trichrome staining, and Wheat Germ Agglutination staining to analyze fibrosis, collagen deposition, and cell morphology within the cardiac muscle. RT-qPCR to analyze the quantity of messenger RNA found in the sample.

Methods

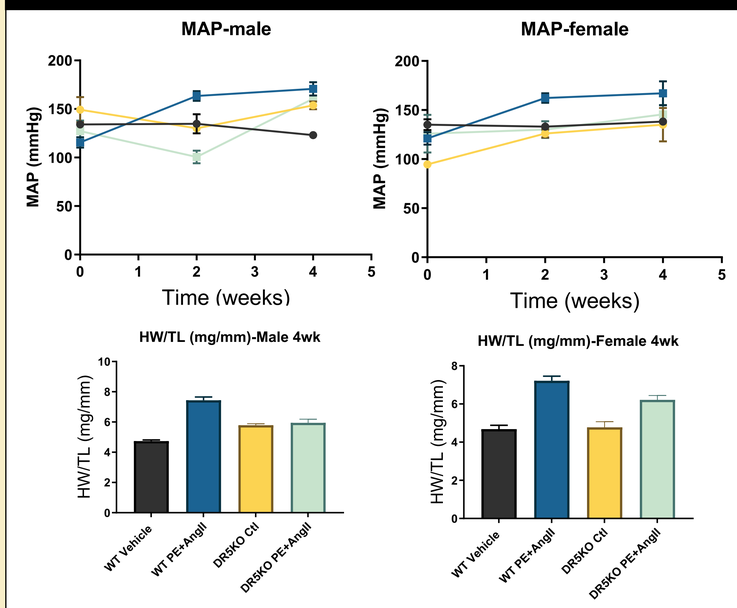

Figure 2. Increase in arterial pressure leads to overloading of the heart which stimulates upregulation of pro hypertrophic genes leading to hypertrophy and fibrosis. Male WT mice given phenylephrine and angiotensin II showed a great increase in mean arterial pressure, compared to the DR5KO mice that had a delayed or buffered initial response. Female WT mice given phenylephrine and angiotensin II showed a great increase in mean arterial pressure, whereas the DR5KO mice remained relatively stagnant. Heart weight to tibia length shows WT mice given angiotensin II had the most hypertrophy and fibrosis female DR5KO mice experienced slightly greater hypertrophy when compared to the males.

Hypertrophic Data

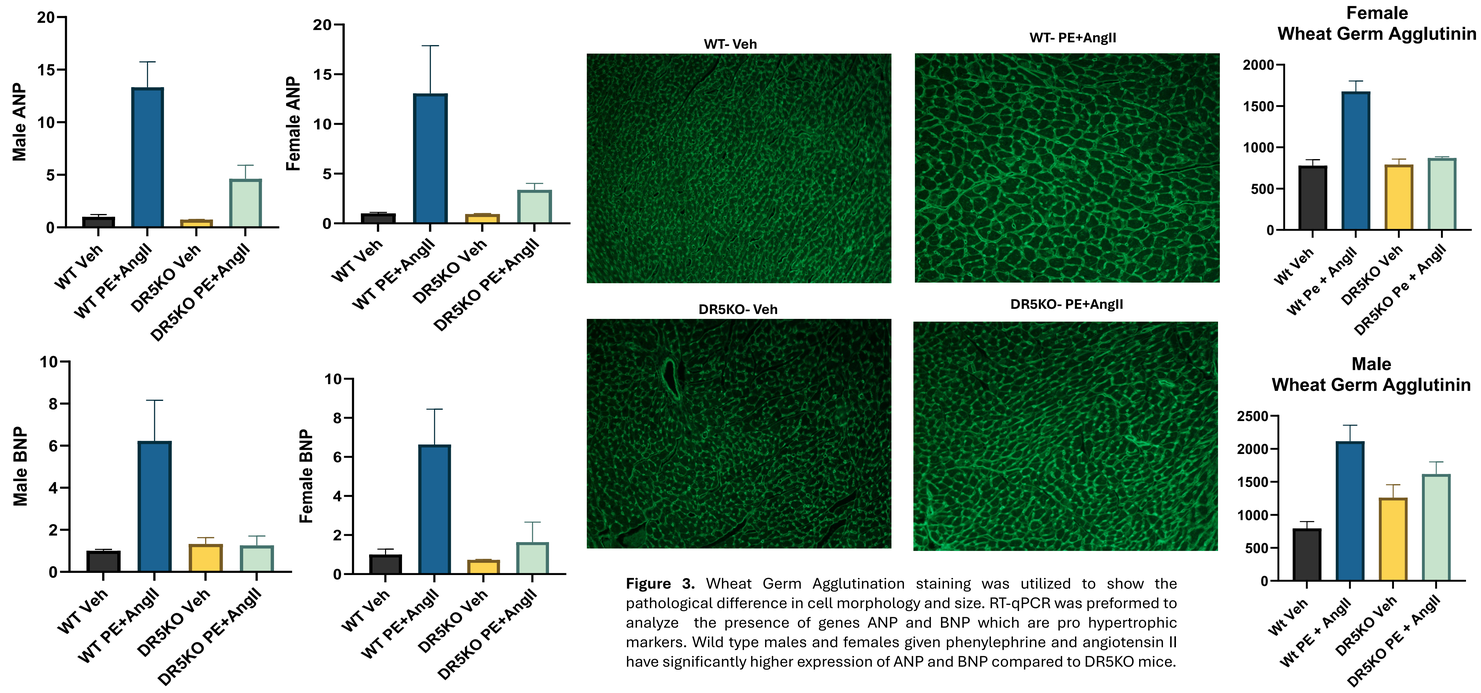

Figure 3. Wheat Germ Agglutination staining was utilized to show the pathological difference in cell morphology and size. RT-qPCR was preformed to analyze the presence of genes ANP and BNP which are pro hypertrophic markers. Wild type males and females given phenylephrine and angiotensin II have significantly higher expression of ANP and BNP compared to DR5KO mice.

Male – Fibrosis

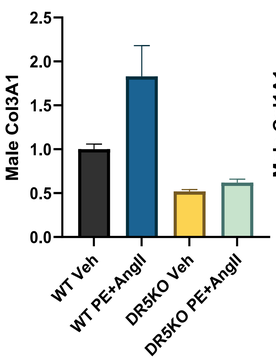

Figure 4. PCR Testing was used to look at genes Collagen 3A1 and Collagen 1A1 to quantify the fibrosis present in the cardiac tissue. Wild type males given phenylephrine and angiotensin II showed the highest levels of Col 3A1 and Col1A1. These genes are activated in response to cardiomyocyte stress and had a diminished response in DR5KO mice.

Female - Fibrosis

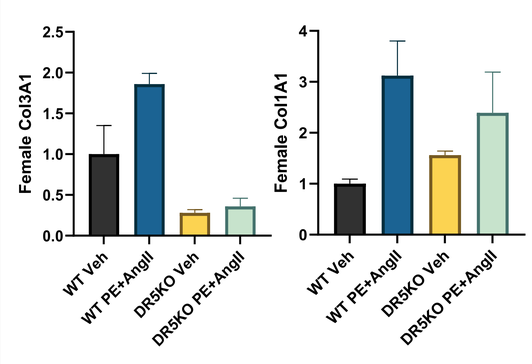

Figure 5. PCR Testing was used to look at genes Collagen 3A1 and Collagen 1A1 to quantify the fibrosis present in the cardiac tissue. Wild type females given phenylephrine and angiotensin II showed the highest levels of Col 3A1 and Col1A1. These genes are activated in response to cardiomyocyte stress and had a diminished response in DR5KO mice

Histology

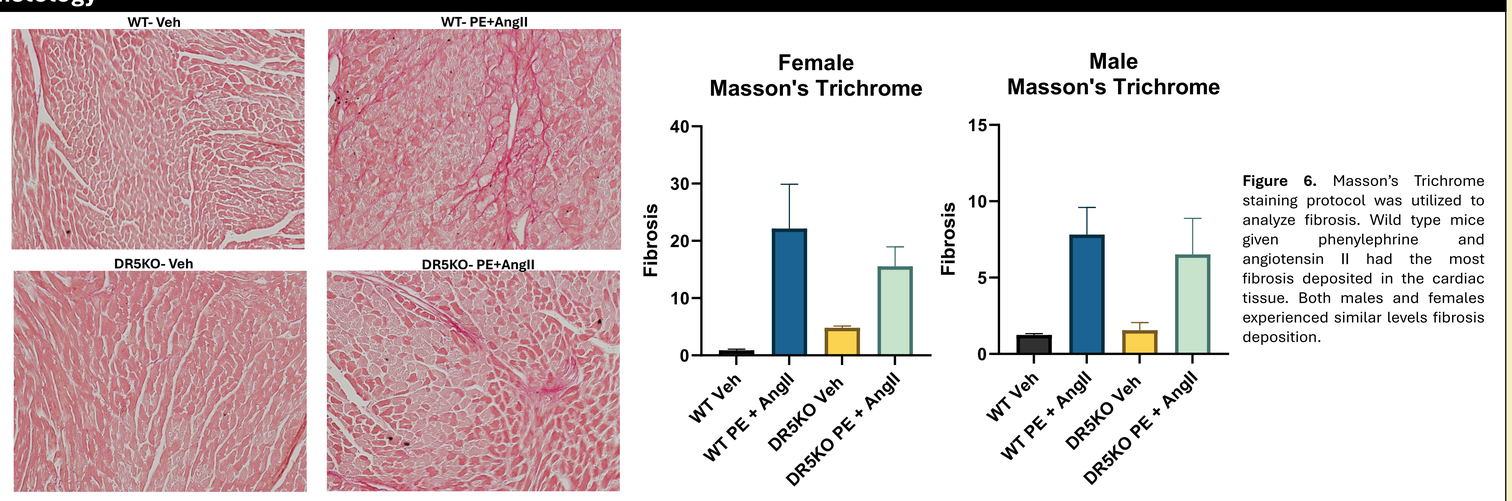

Figure 6. Masson's Trichrome staining protocol was utilized to analyze fibrosis. Wild type mice given phenylephrine and angiotensin II had the most fibrosis deposited in the cardiac tissue. Both males and females experienced similar levels fibrosis deposition.

Fractional Shortening

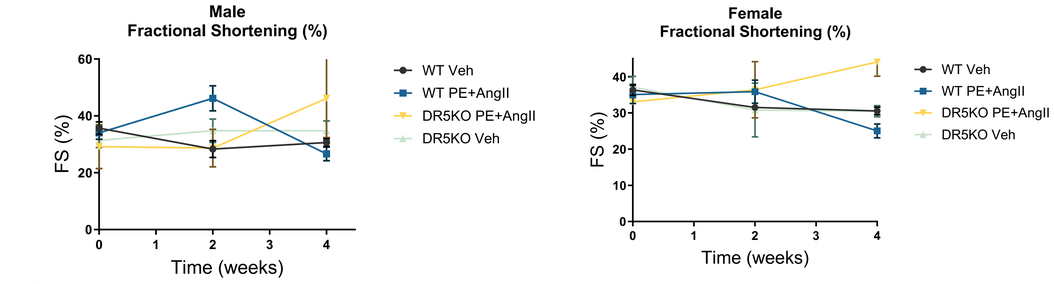

Figure 7. As a result of the cardiac hypertrophy and fibrosis, fractional shortening is shown to greatly decline in wild type mice given phenylephrine and angiotensin II. The cardiac function of DR5KO mice is shown to be unchanging. At first the DR5KO mice look to have an initial blunted response to the hypertensive agents, but then increase their contractility between 14 days and 28 days.

Conclusions

- DR5KO mice have cardioprotective features that decrease the expression of hypertrophic genes.
- DR5KO mice experienced no functional changes in fractional shortening even in the presence of phenylephrine and angiotensin II.
- Male and female mice had comparable results in this study.
- Moving forward studies will investigate systemic and localized causes of blood pressure changes.

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