Novel ‘Dual Hit’ Rat Model of Diabetic Cardiomyopathy

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INTRODUCTION AND AIM

Cardiovascular complications are the leading cause of diabetes-related morbidity and mortality characterized by structural and functional abnormalities of the myocardium. However, there is a lack of translational and robust animal models of diabetic cardiomyopathy that can aid elucidation of mechanisms of action, and support the development of improved therapeutic agents.

Here, we hypothesized that surgically induced diabetes (pancreatectomy) and pharmacological (isoprenaline) driven cardiac stress could accentuate cardiac remodelling and dysfunction resulting in a useful rat model for pre-clinical investigations.

STUDY DESIGN AND GROUPS

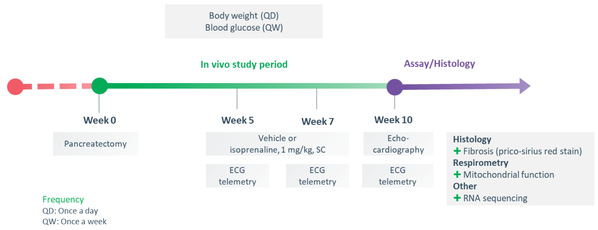


Figure 1 | Male Sprague Dawley rats underwent sham surgery or 90% pancreatectomy (Px). Body weight was recorded daily and blood glucose weekly (4 hrs. fasted). Electrocardiography (ECG) telemetry was measured five, seven and ten weeks after surgery. Cardiac function assessed by echocardiography was measured in week ten. At termination, the left ventricle was isolated for biochemical and histological analyses and next generation RNA sequencing.

Color	Group name	Surgery	Treatment
●	Sham – vehicle	Sham	Vehicle
●	Sham – iso	Sham	Isoprenaline
●	Px – vehicle	Pancreatectomy	Vehicle
●	Px - iso	Pancreatectomy	Isoprenaline

RESULTS

Pancreatectomy resulted in pronounced hyperglycemia

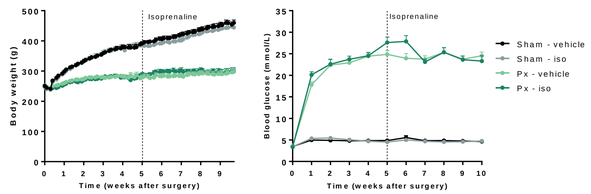


Figure 2 | Daily body weight (*left panel*) and weekly blood glucose (*right panel*). Px rats were considered diabetic and included in study if non-fasted blood glucose was >10.4 mmol/L within 14 days after surgery (one rat was excluded). Data are mean + SEM. n=12-18.

Combining pancreatectomy and isoprenaline caused decreased tibial length and tended towards left ventricular hypertrophy

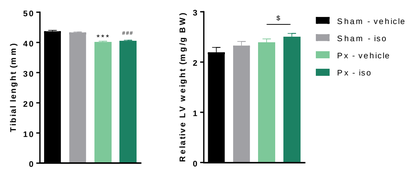


Figure 3 | Absolute tibial length (*left panel*) and left ventricular weight relative to body weight (*right panel*). Data are mean + SEM. Two-way ANOVA ($^{\$}$p<0.05 vs. sham) with Sidak’s post hoc test; ***p<0.001 vs Sham-vehicle; $^{\#\#\#}$p<0.001 vs Sham-iso. n=8-18.

Acute exposure to isoprenaline decreased the R-R interval Pancreatectomy caused elevated R-R interval

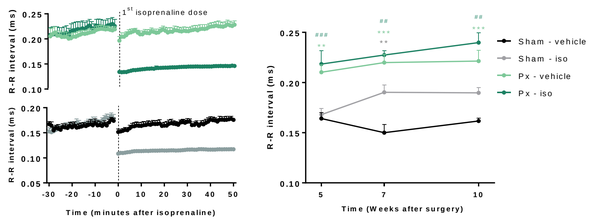


Figure 4 | R-R interval measured immediately before and after first isoprenaline dose (*left panel*). Measurement of R-R interval (*right panel)*. Data are mean + SEM. Two-way ANOVA with Sidak’s post hoc test; **p<0.001, ***p<0.001 vs Sham-vehicle; $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 vs Sham-iso. n=7-8.

Pancreatectomy in combination with isoprenaline caused increased left ventricular diameter and decreased ejection fraction

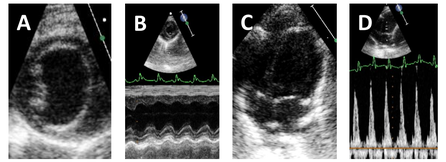


Figure 5 | Cardiac function was examined with a Philips iE33 ultrasonograph and a 12-4 MHz sector transducer. Two-dimensionally guided M-mode of the left ventricle (LV) was taken at the papillary muscle level (A, B). Two-dimensionally guided early LV filling was measured by transmittal pulsed wave Doppler (C, D).

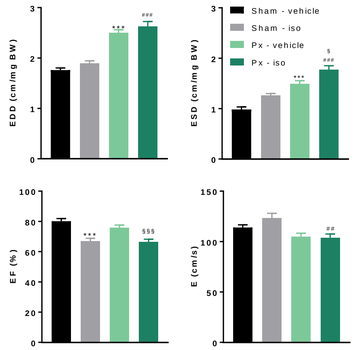


Figure 6 | | Relative left ventricular (LV) end-diastolic and -systolic diameter (EDD, ESD) (*Upper panel*). Ejection fraction (EF) and early LV filling in diastole (E) (*lower panel*). Two-way ANOVA with Sidak’s post hoc analysis; ***p<0.001 vs. sham-vehicle. $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 vs. sham-iso, $^{\$}$p<0.05, $^{\$\$\$}$p<0.001 vs. Px-vehicle. n=12-18.

Gene expression levels indicate pancreatectomy-driven hypertrophy and decreased contractibility

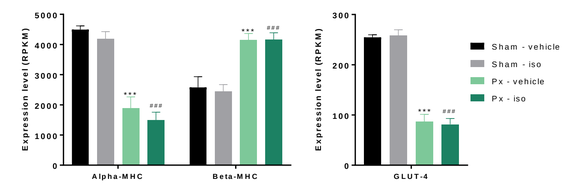
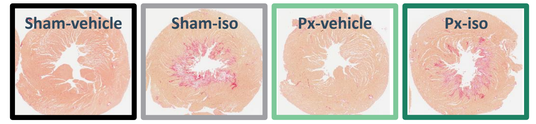


Figure 7 | Left ventricular gene expression levels of alpha- and beta-myosin heavy chain (MHC) (*left panel*) and glucose transporter type 4 (GLUT-4, *right panel*). Data are mean + SEM. Two-way ANOVA with Sidak’s post hoc analysis, ***p<0.001 vs. sham-vehicle, $^{\#\#\#}$ p<0.001 vs. sham-iso.

Isoprenaline caused endomyocardial fibrosis



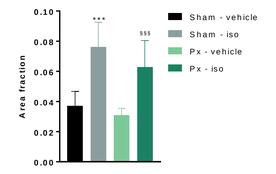


Figure 8 | Left ventricular collagen stained by picro-sirius red (*upper panel*) with quantification of collagen area fraction (*lower panel*). Data are expressed as mean + SEM. Two-way ANOVA with Sidak’s post hoc analysis; ***p<0.001 vs. sham-vehicle, $^{\$\$\$}$p<0.001 vs. px-vehicle. n=8-11.

Mitochondrial function was decreased by pancreatectomy and isoprenaline treatment

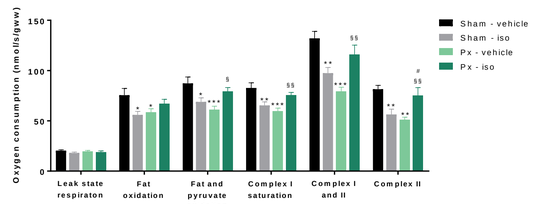


Figure 9 | Mitochondrial function (oxygen consumption) measured using high-resolution respirometry. Saponin permeabilized muscle fiber bundles of the left ventricle were prepared and respiration was measured using substrate-uncoupler-inhibitor titrations. Data are expressed as mean + SEM. Two-way ANOVA with Sidak’s post hoc analysis; *p<0.05, **p<0.01, ***p<0.001 vs. sham-vehicle, $^{\#}$ p<0.05 vs. sham-iso $^{\$}$p<0.05, $^{\$\$}$p<0.01 vs. px-vehicle. n=6-8.

CONCLUSION

The combination of pancreatectomy with isoprenaline treatment exhibited several clinical hallmarks of diabetic cardiomyopathy, incl.:

- Pronounced hyperglycaemia
- Endomyocardial fibrosis
- Decreased left ventricular contractibility and ejection fraction
- Mitochondrial dysfunction (pancreatectomy or isoprenaline)
- Regulation of genes indicative of heart failure and hypertrophy

This model may be useful in the evaluation of cardiovascular effects of novel compounds in the pre-clinical phase of drug development.