A new animal model for investigation of mechanical unloading in hypertrophic and failing hearts: combination of transverse aortic constriction and heterotopic heart transplantation

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Objectives

Previous models to investigate mechanical unloading include large animals (bovine, canine, porcine) and small animal models (rat, mouse), in which heterotopic heart transplantation (hHTx) is performed. While large animal models are expensive and time-consuming, investigation of mechanical unloading in the hypertrophic and failing heart is possible by creating pressure overload by aortic banding or volume overload by artificial mitral regurgitation. On the other hand small animal models are advantageous regarding expenses and handling, whereby hHTx in mice, compared to rat models, put high requirements on microsurgical capabilities. Furthermore, studies of mechanical unloading to date are only performed in healthy or infarcted hearts, not representing the typical dilated heart of end-stage heart failure patients. In this work we present a new and economic small animal model to investigate mechanical unloading in hypertrophic and failing hearts: the combination of transverse aortic constriction (TAC) and hHTx in rats.

Methods

To induce cardiac hypertrophy and failure in rat hearts, three-week old rats underwent TAC procedure (Fig 1). Three and six weeks after TAC, hHTx with hypertrophic and failing hearts in Lewis rats was performed to induce mechanical unloading (Fig 2). After 14 days of mechanical unloading animals were euthanatized and grafts were explanted for further investigations.

Results

Animals presented significant hypertrophy and decreased systolic function 3 and 6 weeks after TAC procedure, respectively (see Figs 3 and 4, see Table 1). When compared to healthy rats left ventricular surface decreased to 5.8±1.0 mm² (vs. 9.6± 2.4 mm²) (p= 0.001) after three weeks of pressure overload with a fractional shortening (FS) of 23.7±4.3 % vs. 28.2±1.5% (p=0.01). Six weeks after application of the clip, systolic function decreased to 17.1±3.2% vs. 28.2±1.5% (p=0.0001) and left ventricular inner surface increased to 19.9±1.1 mm² (p=0.0001). Overall 50 TAC procedures were performed with a survival until scheduled hHTx of 92% (46/50). Two animals died due to intraoperative bleeding and two died due to heart failure. Intraoperative graft survival during hHTx was 80% with 46 performed procedures (37/46). All transplanted organs survived two weeks of mechanical unloading in the recipient abdomen as shown by continuous and regular beating verified by palpation. The mean heart weight loss amounted to 0.2 g in healthy, 0.35 g in hypertrophic and 1.2 g in failing hearts. Histology (see Fig 4) showed typical signs of atrophic myocardial processes such as fibrosis and change in myocyte sizes (see Table 2).

Conclusion

Steady flow MCS found widespread acceptance for the therapy of end-stage heart failure over the last decade. Despite obvious advantages in clinical daily routine, there is a lack of knowledge concerning mechanisms leading to myocardial changes and cardiocytological alterations under mechanical unloading of failing hearts. To date small animal models solely investigate mechanical unloading in healthy or infarcted hearts. Combination of TAC and hHTx in rats offers an economic and reproducible small animal model enabling serial examination of mechanical unloading in a truly hypertrophic and failing heart, representing the typical pressure overloaded and dilated LV, occurring in patients with moderate to severe heart failure. HHTx after TAC is technically demanding when compared to hHTx of healthy hearts. However, once dissection of adhesions is sufficiently trained and ischemia time is minimized graft survival approaches success rate of hHTx utilizing healthy rat hearts.

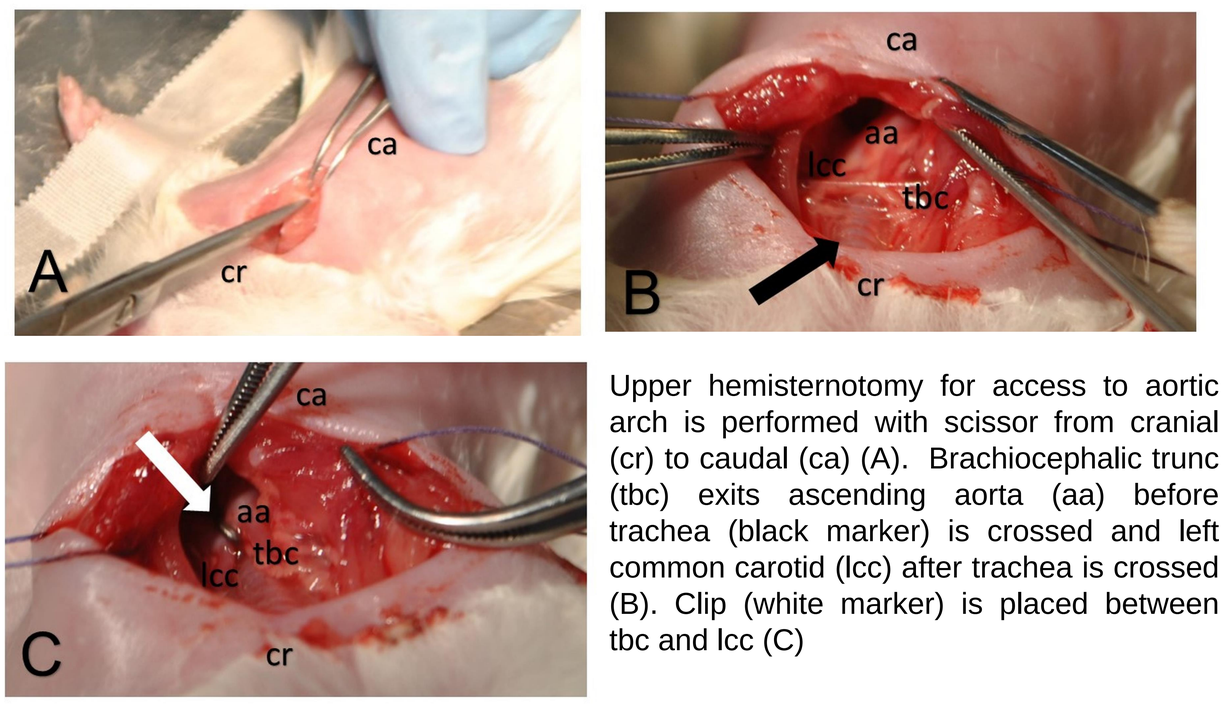
Table 1: Echocardiography data of healthy, hypertrophed and failing rat hearts

	Healthy	Hypertrophed	Failing
Echocardiography			
Ejection fraction (%)	52.1±2.3	33.8±4.5	20.5±16.1
p-value	/	0.0001	0.0001
Fractional shortening (%)	28.2±1.5	23.7±4.3	17.1±3.2
p-value	/	0.01	0.0001
Wall thickness (mm)	1.9±0.1	2.2±0.3	2.1±0.2
p-value	/	0.02	0.02
Left ventricular surface (mm²)	9.6± 2.4	5.8±1.0	19.9±1.1
p-value	/	0.001	0.0001

Table 2: Myocyte cell site after two weeks of mechanical unloading

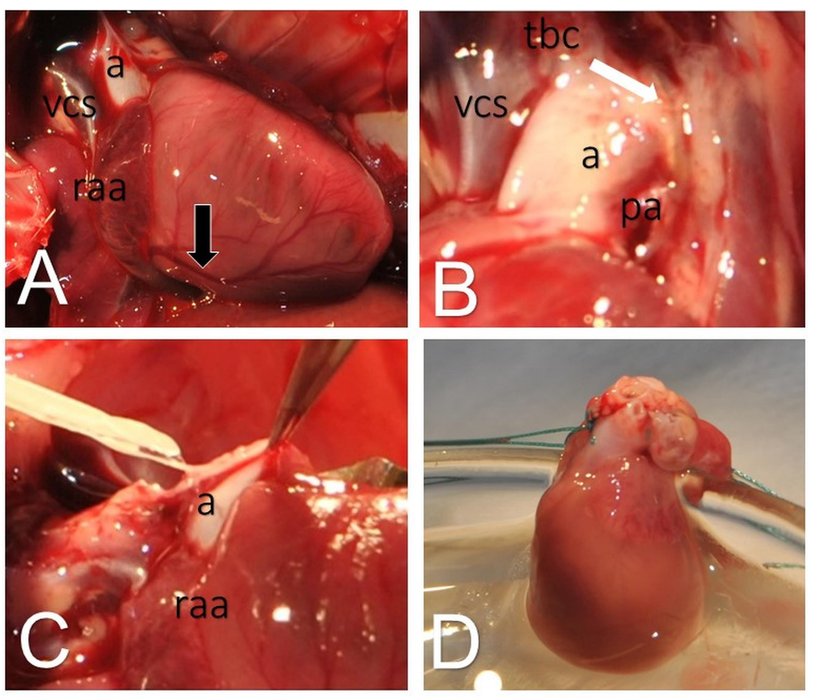
	Healthy hHTx	Hypertrophed hHTx	Failing hHTx
Myocyte cell size (µm)	30.7±6.6	39.8±3.9	38.7±6.4
p-value	0.27	0.01	0.0006

Figure 1: Surgical steps for transverse aortic constriction in three-week old Lewis rats



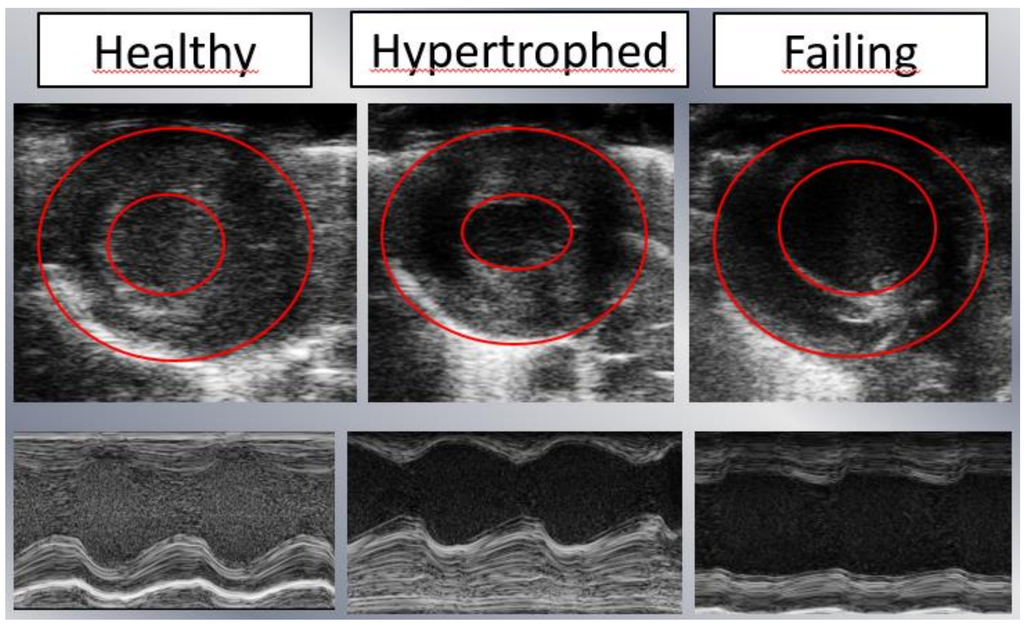
Upper hemisternotomy for access to aortic arch is performed with scissor from cranial (cr) to caudal (ca) (A). Brachiocephalic trunc (tbc) exits ascending aorta (aa) before trachea (black marker) is crossed and left common carotid (lcc) after trachea is crossed (B). Clip (white marker) is placed between tbc and lcc (C)

Figure 2: Technical considerations for heterotopic heart transplantation in Lewis rats with prior transverse aortic constriction



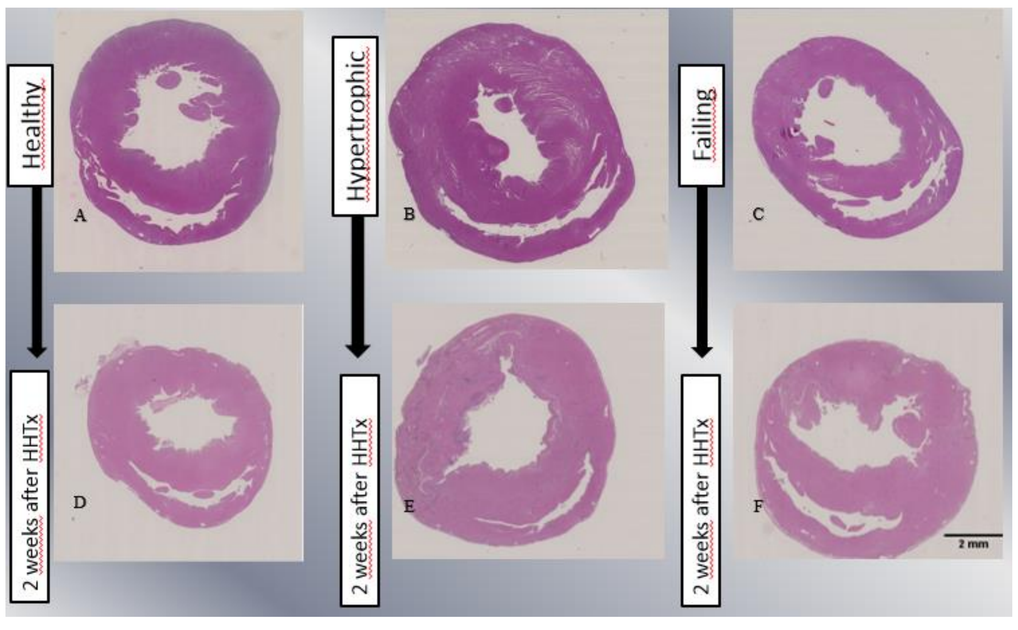
Exposition of hypertrophic heart three weeks after transverse aortic constriction with right coronary artery (black marker), aorta (a), superior vena cava (vcs) and right atrial appendage (raa) (A). View from anterior with aorta (a), clip (white marker) and adhesions of aorta, pulmonal artery (pa), superior vena cava (vcs) and brachiocephalic trunc (tbc) (B). Cardioplegia has to be administered rapidly (C) via a 22 Gauge (0.9 mm) canula leading to collapse of heart function and fading of coronary arteries (D)

Figure 3: Echocardiography of healthy and pressure-overloaded rat hearts



Baseline echocardiography of healthy rat heart in B-Mode (A) and M-Mode (D). Rat heart 3 weeks after TAC with thickened myocardium and reduced end-systolic left ventricular diameter in B-Mode (B) and M-Mode (E). Rat heart 6 weeks after TAC with thinned myocardium and increased end-systolic left ventricular diameter in B-Mode (C) and M-Mode (F). Representative images.

Figure 4: Left and right ventricular heart histology in transversal orientation and haematoxylin staining



A: Healthy heart of 3-weeks old Lewis rat B: Hypertrophic heart of 4-weeks old Lewis rat, 3 weeks after TAC with thickened myocardium C: Failing heart of 7-weeks old Lewis rat, 6 weeks after TAC with thinned myocardium D-F: Healthy heart (D), hypertrophic heart (E) and failing heart (F) of Lewis rat, after 2 weeks of mechanical unloading. Representative images.