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The experimental model of transition from compensated cardiac hypertrophy to failure created by transverse aortic constriction in mice

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ABSTRACT

Background: Transverse aortic constriction (TAC) operation is used as an experimental model of left ventricular (LV) hypertrophy and LV failure in mice. The severity of LV remodeling or failure may depend on the degree of TAC, but is variable among operated animals. Therefore, we tried to identify the optimal diameter of TAC to create this model with ease and high reproducibility.

Methods and results: To produce TAC in C57BL/6J mice (7–9 weeks, body weight 19–26 g, n = 109), a 7–0 nylon suture ligature was tightly tied around the transverse aorta against needles with 3 different diameters (mm); 0.40, 0.385 and 0.375. LV wall thickness, end-diastolic dimension, fractional shortening were measured by echocardiography. At 4 weeks after TAC, no mouse with the 0.400 mm gauge progressed in LV failure. The 0.385 mm pin gauge mouse kept a more survival rate compared with the 0.375 mm (59% vs 48%), representing same efficient in LV failure. With the 0.385 mm pin gauge, hearts of mice remained LV hypertrophy at 1 week after TAC, followed by LV failure at 4 weeks.

Conclusion: TAC with the diameter of 0.385 mm can effectively induce the transition from LV hypertrophy to failure in mice with relatively preserved survival.

1. Introduction

To investigate the effect of drug therapy and the molecular mechanism for heart failure (HF), researchers have used three main models of HF in mice; myocardial infarction (MI) by coronary artery ligation, chronic pressure overload model by transverse aortic constriction (TAC), and chronic volume overload by aortocaval fistula [1]. TAC operation is often used as an experimental model of left ventricular (LV) hypertrophy and HF in mice. In spite of very common model, TAC model has a diversity of the severity of LV remodeling or HF, which has been shown to depend on strain [2], sex [3] and standardizing needle size used for TAC operation [4]. Strain and sex of mice can be determined by study design. However, only the needle sizes need to be adjusted to create an appropriate model for the purpose of study.

In recent studies, needle sizes from 24 to 28-gauge, frequently 27-gauge (0.40 mm outside diameter (OD)), have been selected in TAC operation [5–7]. In the study of HF, we need mice model with severe HF and obvious LV remodeling by TAC keeping high survival rate. However, we actually noticed that some mice remained hypertrophy without the manifestations of HF in surviving mice, others died quickly. The purpose of this study was which size of gauges was suitable for TAC operation from the point of view of survival and severity of HF.

Now pin gauges for industrial use are commercially available, which can vary its size used for TAC operation in 0.005 mm increments. To determine which size of gauges is most suitable for TAC operation, we studied survival and severity of HF after TAC by using three sizes of gauges; medical needle with a 0.400 mm OD, pin gauges for industrial use with a 0.385 mm OD and a 0.375 mm OD.

2. Methods

All experimental procedures and animal care were approved by our institutional animal research committee and conformed to the Animal Care Guideline for the Care and Use of Laboratory Animals of Hokkaido University Graduate School of Medicine and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

2.1. TAC operation

As previously described by Rockman et al. [8], TAC was created in male C57BL/6J mice (7 to 10 weeks old and 19 to 26 g body weight

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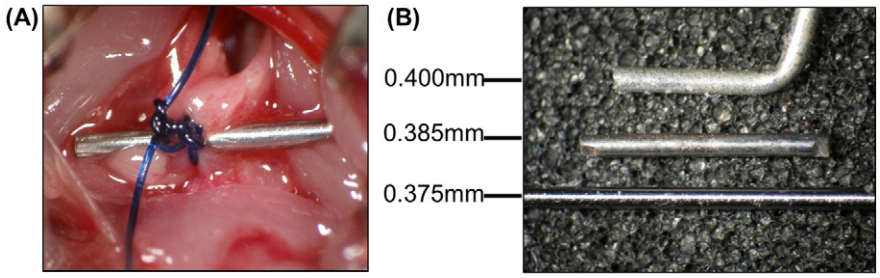

Fig. 1. Photographs of TAC operation. (A) Mice were subjected to TAC operation using the gauge. (B) Three gauges used with TAC operation: top; a 0.400 mm gauge needle, middle; a 0.385 mm pin gauge, bottom; a 0.375 mm pin gauge.

(BW), CLEA Japan, Inc., Tokyo, Japan) by a suture ligature around the transverse aorta. All procedures were performed by a single operator. Briefly, mice were anesthetized with a combination anesthetic, called MMB, via intraperitoneal administration. We mixed 3 drugs; 0.3 mg/kg of medetomidine (Dorbene® Kyoritsuseiyaku Co., Ltd., Tokyo, Japan), 4.0 mg/kg of midazolam (Dormicum®, Astellas Pharma Inc., Tokyo, Japan), and 5.0 mg/kg of butorphanol (Vetorphale®, Meiji Seika Kaisha, Ltd., Tokyo, Japan) [9]. Mice were placed on the pad in the dorsal position, and then the limbs were fixed with surgical tapes. Mice were intubated, the tube connected to experimental rodent ventilators (Shinano Co., Ltd., Tokyo, Japan) under the condition of 120 breaths per minutes and 0.3 ml tidal volume.

The skin from the neckline to the diaphragm was disinfected with 70% alcohol. After thoracic incision with the scissor, the second intercostal space through a small incision with the forceps, the thymus being divided into two pieces, the transverse aorta was exposed with four rib spreaders. A 7–0 nylon (Akiyama Co., Ltd., Yamanashi, Japan) suture ligature was "very tight without deformation of the nylon" tied around the transverse aorta with a thin metal rod to produce a constriction after removal of the rod (Fig. 1A). Three kinds of thin metal rods were used in the present study; the 27 gauge medical needle with a 0.400 mm OD (TERUMO Co., Ltd., Japan), the pin gauges for industrial use with a 0.385 mm OD and a 0.375 mm OD (EISEN Co., Ltd., Shiga, Japan). The picture of these 3 gauges used in the present study was shown in Fig. 1B. Their ODs were confirmed and analyzed with ImageJ software (data not shown). The intercostal muscle, the greater pectoral muscle and the skin were closed by a 4–0 suture, respectively. After spontaneous breathing appeared, mice were extubated and moved into a cage on a pad maintained at 37 °C up to next morning. Sham operation was also performed.

2.2. Experiment 1

Mice were randomly divided into sham (n = 21) and 3 different TAC groups operated by metal rods with a 0.400 mm OD (n = 11), a 0.385 mm OD (n = 29), and a 0.375 mm OD (n = 22). These mice were observed for 4 weeks.

2.3. Survival

The survival analysis was performed in all groups of mice. During four weeks after operation, the cages were inspected daily for dead animals. All dead mice were examined for the presence of TAC.

2.4. Echocardiographic measurements

At four weeks after operation, echocardiographic measurements were performed in surviving mice under light anesthesia with tribromoethanol/amylene hydrate (avertin; 2.5% wt/vol, 8 μl/g ip), as described previously [10]. A two-dimensional parasternal short-axis views were obtained at the levels of the papillary muscles. In general, the best views obtained with the transducer lightly applied to the mid upper left anterior chest wall. The transducer was then gently moved cephalad or caudad and angulated until desirable images were obtained. After it had been ensured that the imaging was on axis, two-dimensional targeted M-mode tracings were recorded at a paper speed of 50 mm/s.

2.5. Organ weights

After an addition of avertin, mice were euthanized by cervical dislocation under deep anesthesia with avertin (2.5% wt/vol, total 10 μl/g ip). Hearts and lungs were then excised and weighed.

2.6. Experiment 2

Another set of mice was divided into control (n = 11), and TAC group operated by metal rods with a 0.385 mm OD and observed for one week (n = 15). These mice were compared with TAC mice operated

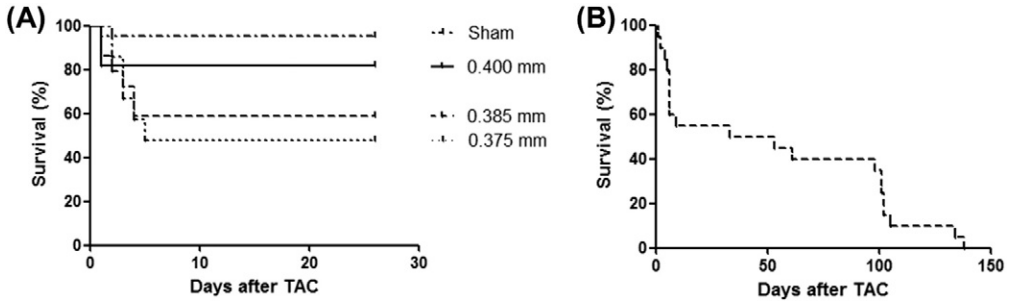

Fig. 2. Survival curves in TAC operation. (A) Mice were subjected to TAC operation with three kinds of gauges and followed for 4 weeks. Sham, n = 21; 0.400 mm, n = 11; 0.385 mm, n = 29; 0.375 mm, n = 22. (B) Mice were subjected to TAC operation with the 0.385 mm pin gauge and followed for 5 months. n = 20.