Simple Methods to Elevate Pulmonary Arterial Pressure by Pre- and Post-tricuspid Shunts in Rats

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ABSTRACT

Background: Arteriovenous shunt in the rat is an extremely useful experimental animal model for investigating cardiac hypertrophy as well as the hemodynamics and endocrine aspects of chronic heart failure. Aims: The present study was to develop 2 pre-tricuspid and 1 post-tricuspid models of arteriovenous shunt to induce right ventricular hypertrophy and increase pulmonary blood flow in growing rats. Methods: In the first model, an arteriovenous shunt was created from the common iliac artery to the inferior vena cava (ICS). The second model was shunted from the common carotid artery to the external jugular vein (CJS). A post-tricuspid shunt (the third model) was made by introducing the right common carotid artery into the right ventricular outflow tract (CVS). Results: Four weeks after the shunt surgery, the pulmonary artery pressure was 14.4 ± 0.5 mmHg in the control group, 15.8 ± 0.8 mmHg in the ICS group, 21.2 ± 0.7 mmHg in the CJS group, and 20.2 ± 1.1 mmHg in the CVS group. The percentage of increasing pulmonary blood flow was 33.0 ± 1.0% in the CJS group and 26.9 ± 1.3% in the ICS group four weeks after shunt operation. The oxygen partial pressure of pulmonary artery blood was 30.9 ± 0.7 mmHg in the control group, 33.6 ± 1.0 mmHg in the ICS group, 43.7 ± 1.4 mmHg in the CJS group and 41.1 ± 2.5 mmHg in the CVS group. The CJS and CVS groups had significant right ventricle hypertrophy. Conclusions: These three models can provide for study of the flow-pressure effect of the right heart and pulmonary circulation.

Key Words: pulmonary hypertension; pretriscuspid shunt, posttricuspid shunt, heart failure, rat

Introduction

Chronic arteriovenous shunt in the rat is an extremely useful experimental animal model for investigating cardiac hypertrophy as well as the hemodynamics and endocrine aspects of chronic heart failure [6,9,11]. The shunt model imposes work overload upon right heart and pulmonary vasculature. Hypertrophy response and significant hemodynamics alterations were obtained in this animal model [7,8, 14].

Flaim et al. (6) created an aortocaval shunt in old Sprague-Dawley rats (639 ± 35 g) under dissecting microscope. This method needs complex microvascular surgery, the entire surgical procedure took 40 min. This procedure has inconvenience of high surgical mortality, unpredictability of shunt magnitude. Garcia et al. (7) reported a simple method of producing aortocaval shunt in adult Sprague-Dawley rats (about 250 g). The aorta was punctured caudal to the left renal artery with an 18 gauge disposable needle, which was advanced into the vessel, perforating the adjacent wall between aorta and vena cava and penetrating the latter. The procedure takes about 10 min. They did not measure the changes of pulmonary arterial pressure and pulmonary blood flow. The unpredictability of shunt magnitude was not evaluated. We have now used native arteries to

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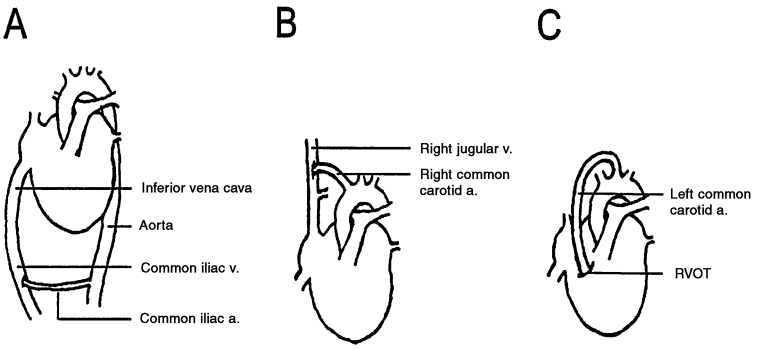

Fig. 1. Schematic drawing showing production of left-to-right shunt models. A. common iliac artery to vena cava shunt (ICS model), B. common carotid artery to jugular vein shunt (CJS model), C. carotid-ventricular shunt (CVS model .

design methods of producing pre- and post-tricuspid left-to-right shunts that were easy, efficient and virtually low mortality in growing rats. We believe that these techniques could be useful to many laboratories interested in rat models of cardiac hypertrophy, heart failure and chronic increasing pulmonary blood flow [5,8].

Methods

Female Wistar rats were housed in constant-temperature facilities, exposed to a 12-h light-12-h dark cycle, and given standard rat chow and tap water at all time. All the animal experiments and care were according to the *Guide for the Care and Use of* Laboratory Animals (published by the National Academy Press, Washington, D.C., 1996). "Laboratory Animal Care Committee" of the National Taiwan University College of Medicine approved the protocol.

(I) Pre-tricuspid Shunt Models

Twenty female Wistar rats (120 ~ 150gm of body weight) were divided into two groups to create pre-tricuspid shunts.

(i) Common Iliac Artery to Vena Cava Shunt (ICS model) (Figure 1,A)

In this group of ten rats, the rats were anesthetized with ketamine (0.6 mg/kg) and the right common iliac artery was dissected through median laparotomy. The blood flow of right common iliac artery was measured with transonic flowmeter (Transonic Systems Inc. T106, NY). After proximal clamping, the right common iliac artery was ligated distally and amputated just before the bifurcation. A 0.2mm hole was made in the right common iliac vein under an operative microscope, and then without suturing, the proximal stump of the right common iliac artery was introduced into the hole of the right common iliac vein and up to the inferior vena cava. The right common iliac artery stump was compressed with a peritoneal fat to stay firm on the spot.

(ii) Common Carotid Artery to Jugular Vein Shunt (CJS model) (Figure 1,B)

Ten rats of this group were anesthetized with ketamine (0.6 mg/kg) and the right common carotid artery and right external jugular vein were dissected. The blood flow of right common carotid artery was measured with transonic flowmeter. The proximal site of the right common carotid artery was clamped and the distal site of the artery was ligated. The right common carotid artery was amputated just before the bifurcation. A 0.2mm hole was made in the right external jugular vein under an operative microscope and the proximal stump of the right common carotid artery was rotated and introduced into the hole in the right external jugular vein.

After the procedure in these two groups, the shunts were compressed with cotton for hemostasis and the proximal clamps of the arteries were released simultaneously. Ten minutes later, the cotton was removed and the patency of shunts was assessed visually by the presence of arterial blood in the vena cava or jugular vein. The surgical wounds were sutured with 5-0 Nylon suture.

(II) Post-tricuspid Shunt Model

The surgical procedure of the post-tricuspid shunt, the carotid-ventricular shunt (CVS model) (Figure 1,C) was relative complication. Ten Wistar rats (about 150gm body weight) were anesthetized with pentobarbital sodium (40 mg/kg, ip) and intubated through the oral cavity into the trachea. The rat was placed on an animal thermostat plate with a rectal thermometer to monitor rectal temperature. The rat was ventilated with a respirator (tidal volume 1 mL/ 100mg body weight, respiratory rate 60 breaths/min). The left common carotid artery was dissected and the

Table 1. Characteristics Compared between Pre- and Post-tricuspid Shunts in Rats

	BW (gm)	PAP (mmHg)	PO_2 (mmHg)	RV/LV+S
Normal (n = 10)	198.5 ± 3.1	14.4 ± 0.5	30.9 ± 0.7	0.268 ± 0.004
ICS (n = 9)	195.5 ± 2.9	15.8 ± 0.8	33.6 ± 1.0	0.286 ± 0.007
CJS (n = 9)	192.8 ± 4.0	21.2 ± 0.7**	43.7 ± 1.4**	0.315 ± 0.008**
CVS (n = 6)	202.5 ± 7.9	20.2 ± 1.1**	41.1 ± 2.5**	0.311 ± 0.008**

ICS, iliac artery to vena cava shunt; CJS, common carotid artery-to external jugular vein shunt; CVS, common carotid artery-to right ventricular shunt; BW, body weight; PO_2, oxygen partial pressure; RV/LV+S, weight ratio of right ventricle and left ventricle + septum

**p < 0.01 when compared to normal group. Data are shown as means ± SEM.

proximal site of the artery was clamped and the distal site of the artery was ligated. The left common carotid artery was amputated just before the bifurcation. The whole rat was wrapped with a bag of crashed ice to cool down the rectal temperature to 20°C in order to slow down the heart rates. A partial upper sternotomy was performed and the right ventricular outflow tract (RVOT) was explored carefully with a pericardiotomy. A PE-10 tube was directly punctured into the right ventricle outflow tract as a mark. The proximal stump of the left common carotid artery was rotated and introduced into the RVOT through the puncture mark. The RVOT was compressed with cotton for hemostasis and the proximal clamp of the carotid artery was released simultaneously. Ten minutes later, the cotton was removed and the patency of the shunt was checked with the pulsation of the carotid artery. Then, the sternal wound was closed with 3-0 Dexon suture. The rat was warmed up to rectal temperature 37°C.

After the surgical procedure, all rats were allowed to wake. Lincocin (30 mg/kg/day) and karamycin (15 mg/kg/day) were intramuscularly administered for 3 days. The rats received care, and with food and water freely available at all times. Four weeks after surgery, the shunted rats and normal rats were brought to the operating room and weighed. The rats were then anesthetized, and on respirator ventilation, a midline sternotomy was performed. The blood flow of the pulmonary artery was measured with a transonic flowmeter as previously described. The blood flow change in the pulmonary artery was recorded before and after the shunt was clumped. Then, the pulmonary artery pressure (PAP) was measured by direct puncture of the right ventricle into the pulmonary artery, and recorded the pressure on a polygraph.

Immediately after measuring the pulmonary artery pressure, a blood sample (0.3 mL) was taken from the pulmonary artery. This sample was utilized to determine oxygen partial pressure (PO_2, mmHg) using a blood gas analyzer (Rapidlab800, Chiron Diagnostics Corporation, East Walpole, MA, USA). Then, the hearts were removed and the right ventricle and the left ventricle with septum were individually weighed to calculate the ratio of right ventricle weight to left ventricular plus septum weight (RV/LV+S).

The results are expressed as means ± standard error of mean (SEM). Newman-Keuls test was used to determine statistical significance. P values smaller than 0.05 were considered to be statistically significant.

Results

In rats (about 150gm), the blood flow of the common carotid artery was 8.3 ± 0.4 mL/min, and the common iliac artery flow was 3.8 ± 0.2 mL/min. The common carotid artery flow was greater than that of the common iliac artery in rats. The common iliac artery is more variable in blood flow but the common carotid artery flow is relatively constant.

All the animals appeared healthy and no significant difference in final body weight. There was a rat with shunt occlusion respectively in ICS and CJS group. In CVS group, there were two rats with sternal wound infection and two rats with shunt occlusion. There was no surgical mortality in these three groups. Table 1 shows changes in the PAP and right ventricular hypertrophy (RVH) in the experimental rats 4 weeks after shunt operation. The pulmonary artery pressure was significantly elevated in CJS group (21.2 ± 0.7 mmHg) and in CVS group (20.2 ± 1.1 mmHg). Right ventricular hypertrophy was found in the CJS group (0.315 ± 0.008) and in the CVS group (0.311 ± 0.008). The blood oxygen partial pressure of pulmonary artery was increased in the CJS group (43.7 ± 1.4 mmHg) and in the CVS group (41.1 ± 2.5 mmHg).

The percentage of increasing pulmonary flow was 33.0 ± 1.0% in the CJS group and 26.9 ± 1.3% in the ICS group four weeks after shunt operation.

Discussion

Evidence for significant hemodynamics alterations and structural changes secondary to chronic arteriovenous shunt has already been well documented (6,9,14). These methods all increase pulmonary vascular resistance and shear stress on the vascular wall. In this study, we did not intend to investigate the pathophysiological mechanism that may induce elevated preload to right ventricle and pulmonary circulation, and high output cardiac failure. However, we have devised relatively simple techniques of generating pre-tricuspid and post- tricuspid shunts in rats. Our models, like other previous models (7,9), demonstrate that the shunts can induce right ventricle hypertrophy and increased pulmonary blood flow in rats.

Not all species demonstrate a rise in pulmonary artery pressure in response to a chronic increase in pulmonary blood flow. In the dog, when the pulmonary blood flow is increased by a pre-tricuspid shunt, the pulmonary arterial pressure may stay within the normal range for many years (16). Similarly, months or years after pneumonectomy, the pulmonary artery pressure at rest was not found to enhance (4). Like other aorta-to-cava shunt models, although our ICS group can increase pulmonary blood flow 26.9 ± 1.3%, the flow-pressure effect on the pulmonary circulation is limited. The increased flow from the common iliac artery seems to be well tolerated by the pulmonary vascular bed (2). The CJS model could induce more flow-pressure effect on the right atrium, right ventricle and pulmonary circulation. The common carotid artery had a larger and more constant shunt flow than that of the common iliac artery. An increase in pulmonary blood flow and oxygen partial pressure can alter shear stress effect on pulmonary circulation.

The mechanical force may be an important stimulus to remodeling of the pulmonary vasculature. The mechanism to elevating pulmonary artery pressure in our shunt model is still not known. However, that the increased pulmonary blood flow could induce effective shearing stress in the pulmonary vasculature is currently believed to be a common and critical factor for the initiation of vascular wall changes (1, 12,15). Pulmonary blood oxygenation may play a factor because the vascular response to nitric oxide (NO) can be inhibited or abolished by hemoproteins and superoxide radicals (3). Heller et al. reported that the reduction of the Hb-induced pulmonary hypertension by NO-donors points toward the inactivation of NO by free hemoglobin (10). Liu et al. reported that in small, porcine, isolated pulmonary arteries, intralumenal flow increases the production of NO but this is obscured by the generation of superoxide, which causes vasoconstriction (13).

Normally, smooth muscle extends with age into arteries more peripheral within the acinus; the wall thickness of the normally muscular arteries decreases, and there is a decreasing ratio of alveoli to arteries, indicating an increase in the number of arteries. In a child with a ventricular septal defect, abnormalities in all three features of normal growth and remodeling may be seen, that is, “precocious” extension of muscle into peripheral arteries, medial hypertrophy of muscular arteries, and a reduced concentration of arteries, i.e., increased alveolar arterial ratio (17). Therefore, growing rats may provide a more suitable experimental animal model of chronic high-flow pulmonary vascular remodeling.

Finally, the common iliac artery to vena cava shunt can increase pulmonary blood flow about 25%, but no significant effect to elevate pulmonary artery pressure and to induce right ventricular hypertrophy. This ICS model is adequate for study preload effect, but not pressure factor, on right atrium and right ventricle. The common carotid artery to jugular vein shunt can increase pulmonary blood flow and oxygen partial pressure. This CJS model also can induce right ventricular hypertrophy and elevate pulmonary artery pressure. In CJS model, the shunt is relatively close to the heart, and it is adequate for study volume and pressure factors on right atrium, right ventricle and pulmonary vasculature. The CVS model was designed to induce a post-tricuspid shunt, which had a flow-pressure effect on the right ventricle and lung, but not on the right atrium. This model had similar effect on the right ventricular hypertrophy and increased pulmonary blood oxygen partial pressure, but the shunt flow was relatively unpredictable after the healing process at the RVOT site.

In conclusion, we have devised these three simple and efficient models of inducing left-to-right shunts, which we believe may be useful to those laboratories interested in investigating high output heart failure and cardiac hypertrophy.

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