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Two different rat models for cardiac hypertrophy by constriction of ascending and abdominal aorta

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Abstract

Atherosclerosis is one of the important life style diseases of human beings. There are several animal models in vogue for studying the patho-physiology of Atherosclerosis. It is usually done by inducing pressure overload of aorta. In this study we attempted two different murine models by constriction of thoracic aorta (ascending aorta) and by constriction of abdominal aorta, using metal clips. It was observed that thoracic aorta banding requires expertise, expensive equipment and an increased mortality rate compared to abdominal aorta banding. But the time period for the development of cardiac hypertrophy was lesser in thoracic method (2-4 months) compared to abdominal aorta approach (20-24 months).

Key words : Atherosclerosis, cardiac hypertrophy, rats, aorta

Introduction

Animal models are increasingly used for studying various human diseases. Cardio vascular diseases are on a rise and many are attempting to develop suitable animal models for studying cardiovascular disease biology especially for atherosclerosis. Atherosclerosis is a multifactorial slow developing disease which is very prevalent and ubiquitous. The complexity and chronicity of disease mechanism warrants elaborate studies with suitable animal models. The animal models for atherosclerosis can be created by inducing pressure overload of aorta (Berry *et al.,* 2007). The most commonly used techniques for inducing overload are by constriction of ascending aorta (Feldman *et al.,* 1993), constriction of supra renal abdominal aorta (Yamamoto *et al*., 2007) or by constriction of transverse aorta (DeAlmeida *et al*., 2010).

Rats with aortic constriction will develop left ventricular (LV) hypertrophy as a consequence of persistently increased after load due to aortic stenosis which will eventually lead to cardiac hypertrophy and progressive heart failure. Aortic constriction can be induced by using metal clip or suture materials. This article summarizes two different techniques for inducing aortic pressure overload by banding thoracic aorta and abdominal aorta.

Materials and Methods

Materials

Animals

Forty male Wistar rats (150-200g body weight; 6-8 weeks age) were divided into two groups with 20 animals each. One group had undergone ascending aorta constriction and the other group was subjected to abdominal aortic constriction.

Surgical materials

The materials required for the surgery are listed below.

Item	Specifications
Ligating clip applier	WECK, USA (shown in Figure 1)
Titanium ligating clip	Small, HORIZON; colour code- yellow (shown in Figure 1)
4-0 Silk suture	Ethicon black braided suture
4-0 prolene suture	Ethicon sterilized non absorbable suture
Scissors	Straight, 6 inch
Thump Forceps	Pointed
Thump Forceps	Rat toothed
Artery forceps	6 inches, straight
Curved haemostat	3.5 inches, mosquito type
Suture needle	Curved and straight, with cutting edge and round edges
Needle holder	6 inches
BP handle and blade	size 11
Tracheal cannula	Cannula designed specifically for rats

Special equipment for constriction of ascending aorta

Rodent ventilator (CWE, SAR- 830/AP, USA)
Inhalation anaesthesia systems (VetEquip, USA)

Other materials

Other materials required for the surgery are animal clipper, heating pad, sterile gloves, drugs like buprenorphine, oxytetracycline, betadine, Isoflurane and a light source.

Methods

All procedures were approved by Institutional animal ethics committee (IAEC) of Rajiv Gandhi Centre for Biotechnology (RGCB). All the surgical instruments were sterilized by

autoclaving at 121 °C for 15 min. Instruments were wiped dry with 70 % alcohol prior to the surgery. All the standard aseptic precautions were taken prior and post surgery.

Anaesthesia

Rats were anaesthetized in the induction chamber with 5 % isoflurane mixed with 0.5 -1.0 L/min 100 % oxygen; anesthesia was maintained with 2 % Isoflurane (VetEquip, USA). Depth of anesthesia was determined by checking the tail and pedal reflexes.

Constriction of Abdominal Aorta

The abdominal aortic constriction was performed as per the method of Ganguly *et al*., (1989) and Yamamoto *et al*., (2007) with minor modifications. Briefly, the abdominal region of the rat was shaved and prepared aseptically using 70 % alcohol. A midline skin incision was made on the mid-ventral area of abdomen. Skin, muscle and peritoneum were separated and carefully located the abdominal aorta with the help of light source. Aorta was ligated to about 60 % of original lumen size using titanium clip. After this, muscle layer and skin were closed using silk suture material. The important steps of the procedure are presented in Figure 2.

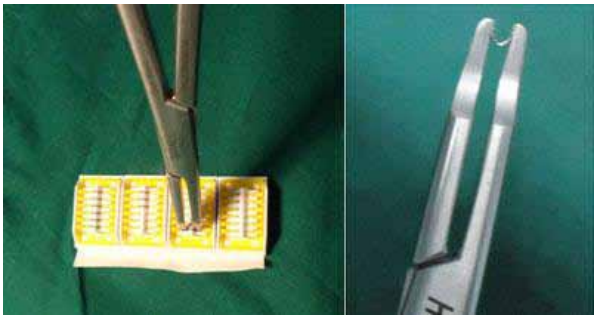
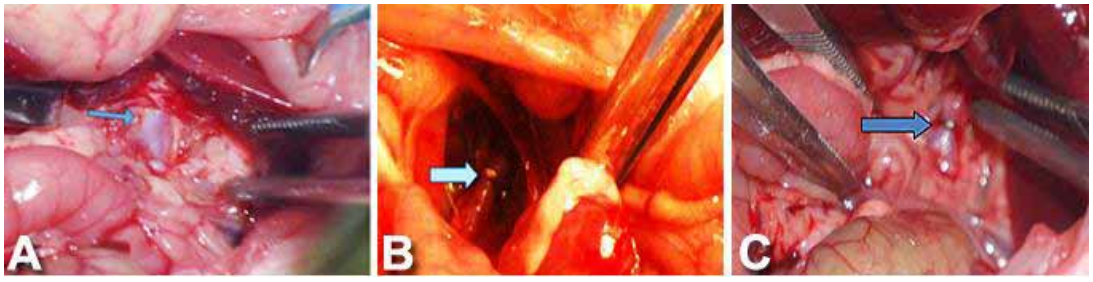


Figure 1: ligating clip applier with ligating clip

Figure 2: A. Skin and peritoneum were separated to locate the abdominal aorta. **B**. Arrow indicates abdominal aorta **C**. Abdominal aorta was constricted using titanium clip. Arrow indicates the clip and constricted portion of abdominal aorta.



Constriction of ascending aorta

The ascending aortic constriction was done as per the method described elsewhere (Turcani and Rupp, 1997; Turcani and Rupp, 2000; Feldman *et al*., 1993). When the rat is properly anesthetized, the chest region was thoroughly clipped with the animal clipper and skin sterilized by applying 70 % alcohol. For stabilizing the respiration of rat subsequent to opening of thorax, a tracheotomy and intubation should be performed prior to opening the thorax.

Tracheotomy and Tracheal intubation

Using BP blade, a small incision was made parallel to the trachea and carefully separated the tissue around trachea in order to expose the trachea. A tie was inserted underneath the trachea and gently raised the tie upward and a half way incision was made between cartilage rings. Tracheal cannula was placed in the trachea which in turn was connected to a rodent ventilator (CWE, USA) with the following settings; respiratory rate - 50 breaths/minute, tidal volume - 1.7 ml and inspiration time - 0.60 seconds.

Procedure for constriction of ascending aorta

An incision was made on the left chest wall between the second and third ribs. The superficial and deep muscles covering the ribs were cleared to expose the intercostal muscle. A 1.5 cm wide incision was made between the ribs by cutting intercostal muscles. The ribs were retracted and ascending portion of aorta was located. Small sized titanium clip was placed with the help of applicator so that aorta will be constricted to 60-70 % of its original diameter; taking care not to include the vena cavae and pulmonary artery inside the clip, which courses adjacent to aorta.

After ligation, second and third ribs were approximated and sutured with 4-0 prolene suture. Simultaneously, ventilator was paused in order to re-inflate the lungs. Air was aspirated from the pleural cavity with a syringe attached to the cannula to restore normal intra-pleural pressure. Muscle layers were apposed with 4-0 prolene sutures and the skin was closed with 4-0 silk sutures in interrupted manner. Tracheal cannula was removed once spontaneous breathing was re-established. Tracheal opening was closed with prolene suture and the skin layer with silk suture. Animal was allowed to recover by lowering the anaesthesia gradually to the off position. The various steps involved in the procedure are demonstrated in Figure 3.

Post operative care

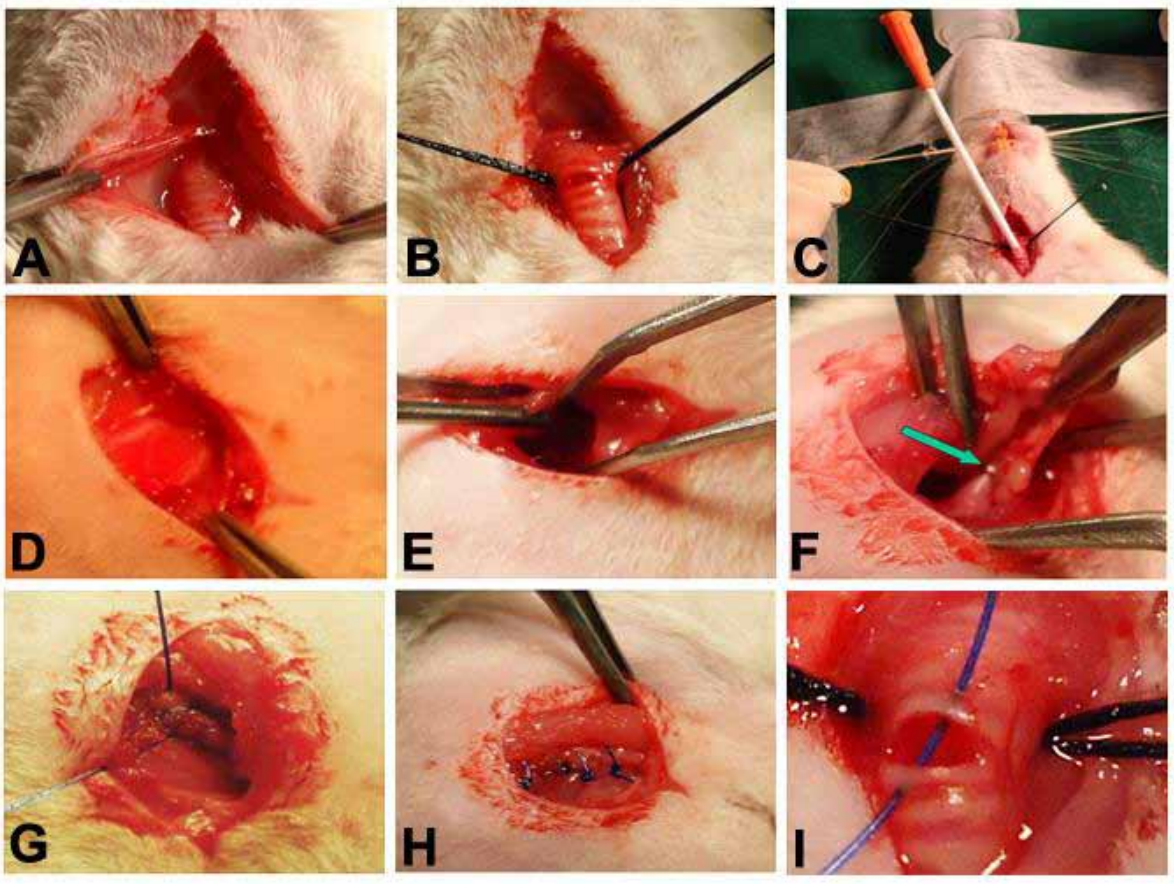


Figure 3: A. Exposing Trachea **B**. Raised trachea with incision **C**. Trachea with canula **D**. Exposing rib cage **E**. Ascending aorta was constricted using the titanium clip **F**. Arrow indicates the clip and constricted portion of ascending aorta **G**. Closing the ribs with prolene suture **H**. Muscle layers were apposed with prolene sutures **I.** Closing the trachea with prolene sutures

For post-operative analgesia, rats were injected with buprenorphine (0.1 mg/kg) intraperitoneally. Oxytetracycline (500 mg/L of water) was mixed in drinking water and fed to the rats for a period of 7 days.

Results and discussion

We tried two methods for creating animal model for atherosclerosis - constriction of abdominal aorta and ascending aorta. Constriction of abdominal aorta was much easier to perform than ascending aorta. No sophisticated equipment or high expertise is needed in case of abdominal aortic constriction. Mortality rate was very less (5%) and if at all happens; it might be due to over ligation of aorta. The skill of banding the abdominal aorta can be easily acquired. But the disadvantage of this model was that the time required to develop hypertrophy was very prolonged when compared to the other model. On an average it takes 20 to 24 weeks to develop a significant level of cardiac hypertrophy and associated pulmonary changes. Hence, it is suitable for studies which are chronic in nature.

The method of constriction of ascending aorta was more difficult to perform as it involves the opening of the thorax. Opening of thorax necessitates micro ventilator to support lungs during the surgical procedure. It also requires very good surgical skills and experience to perform the procedure with increased survival rate. The thoracic aorta lies very close to pulmonary artery and the venacavae and there was every chance that those vessels will also get incorporated within the clamp, if done improperly. Also, care should be taken to avoid the tampering of pericardium. We have to give some extra care during post operative care as chance for pain much more likely and should be periodically observed. Use of analgesics was strictly recommended. Chance for infection was also higher. Mortality will be higher in this case when compared to abdominal aortic constriction. We were having a higher mortality rate of 25% in this method compared with 5% in abdominal aortic banding. But the method has got an advantage of getting the cardiac and pulmonary changes within a short span and is suitable for studies which require an acute progression. We were able to detect cardiac changes in an average time period of 8 weeks. 8 weeks post aortic banding; rats will develop LV hypertrophy, consistent with "compensated hypertrophy" with maintenance of LV chamber size. By 16 weeks, obvious signs of heart failure was evident that are associated with LV dilation and systolic dysfunction.
Animal models of diseases will allow researchers to elucidate the different stages, the progress and mechanisms involved in the pathogenesis of a disease. It is essential to select the suitable animal for creating an animal model. Rats are very similar to human beings and thus can serve as an excellent animal model considering their small size, easier handling and management. There have been many previous reports of rats being used for cardiac disease biology studies of hypertension and cardiac hypertrophy (Doggrell and Brown, 1998).

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