Myocardial Infarct in the CD Rat

Charles River Laboratories, 251 Ballardvale St. Wilmington, MA 01887

charles river
accelerating drug development. exactly.

Abstract

Coronary artery disease is the leading cause of death worldwide. The primary cause of coronary heart disease and subsequent heart failure is atherosclerosis of coronary arteries leading to their occlusion and subsequent ischemic damage to the heart muscle. A number of animal models that utilize ligation of the coronary artery have been described, including large species such as the swine and canine models, as well as rodent models. Charles River Surgical Services performed the following study to characterize our rat myocardial infarction model. The goal of the project was to document the expected size of ischemic lesion following ligation of the left anterior descending coronary artery (LAD) in the CD rat. Forty-two male CD® rats [Crl:CD(SD)] weighing 175-200g, underwent the surgical ligation of the LAD coronary artery, followed by euthanasia and measurement of the resulting ischemic lesion. The results of this study indicate that ischemic lesion comprising 20-30% of total cardiac tissue can be reliably produced in at least 70% of the animals that underwent surgery.

Introduction

Coronary artery disease (ischemic heart disease) is the leading cause of death worldwide, and accounts for 12.8% of annual deaths according to the World Health Organization's website (http://www.who.int/mediacentre/factsheets/fs310/en/index.html). In most cases, myocardial infarction (MI) is a consequence of coronary artery disease where the subsequent acute cardiac failure results in high rates of morbidity and mortality. In humans, the majority of myocardial infarcts result from thrombotic occlusion by arteriosclerotic plaques. In experimental animal models, this occlusion phenomenon can be mimicked by ligation of one of the coronary arteries. Models of coronary arteries ligation have been developed in several different species. Early studies in large animals, particularly dogs, were published in the beginning of the 19th Century.[1-3]

The expense and practical demand of large animal surgical facilities severely limits the extent of such studies. Smaller animal models were therefore developed, and the rat model of myocardial infarction by coronary artery occlusion was first published in 1954.[4] A primary challenge with the LAD rodent model is the difficulty associated with placing a ligature around the LAD in a rapidly beating rat heart. This challenge contributes to variability in placement of the suture, and thus placement of the LAD occlusion, which directly affects the size of the infarcted muscle downstream from the occlusion.

The objective of this study was to characterize the model, to standardize the expected outcomes, i.e. infarct size, as well as to help Surgical Services refine the training of surgeons to produce a consistent size of cardiac infarct for subsequent use by cardiovascular researchers. This report provides summary information on the outcome of the study.

Materials and Methods

Animals

Forty-two male CD® rats [Crl:CD(SD)], weighing 175-200g, were utilized. The animals were transferred to the surgical barrier, group-housed in filter top polycarbonate cages, and acclimated for 1-2 days prior to surgery. Post-surgery, the animals were group-housed, six per cage, in the filter top polycarbonate cages for 20-24 hours before undergoing euthanasia for heart analysis. The animals were kept on Beta Chip bedding during the entire process, supplied with filtered UV sterilized water in bottles, and fed LabDiet 5L79 rodent chow. This study was conducted under a Charles River IACUC approved protocol, and was performed within a AAALAC, International accredited facility. All animals were of the VAF/Plus® health status.

Surgical Procedure

Animals were anesthetized with a cocktail of ketamine (43 mg/kg IP) and xylazine (8.7 mg/kg IP) then aseptically prepped for surgery. The left chest wall was shaved and the skin prepared using alternating applications of povidone iodine solution (Betadine™) and 70% isopropyl alcohol. A preoperative dose of buprenorphine (0.02 mg/kg SC) was administered and the animal was transferred to a HEPA-filtered laminar flow hood, placed on a heated surgical surface, and then draped for surgery. Animals were intubated and positive pressure ventilation was provided by a ventilator (Harvard Apparatus Inspira ASV Model 55-7059). Tidal volume and rate were determined by preprogrammed settings based on animal weight. A 3 cm transverse incision was made between the fourth and fifth intercostal spaces. The heart was then exposed and the LAD coronary artery was ligated between the pulmonary cone and the left auricle using 5-0 silk suture with a no. 4 ½ circle taper point needle. The intercostal space was then closed with 4-0 prolene suture and the skin incision closed with wound clips. The procedure was completed in approximately 60 minutes, after which animals were placed in a heated oxygen chamber, and once ambulatory, transferred to their holding cages for recovery.

Measurement of Infarct Size

The use of vital stains, such as 2,3,5-triphenyltetrazoliumchloride (TTC) to measure myocardial infarct size following coronary artery ligation in experimental animals, is a widely accepted technique.[5-7] Animals were euthanized by CO2 asphyxiation at 20-24 hours post-operatively. Hearts were dissected free of the thoracic cavity and rinsed with Phosphate Buffered Saline (PBS) at 4°C to remove excess blood. The hearts were then placed into –20°C freezer for approximately one hour to facilitate tissue processing. To ensure uniform thickness of the tissue sections, hearts were positioned in a rat coronal brain matrix (Braintree Scientific model BS-AL-6000C) and cut into six, 2 mm cross-sections, using a razor blade.

Heart sections were then placed into a Petri dish and stained with 1% TTC solution (VWR Scientific 90002-202) in an incubator at approximately 37°C for 20 minutes.

Following staining, the slices were fixed in 10% Neutral Buffered Formalin for 20 minutes. Infarcted areas were visible as a pale discoloration (TTC negative), while viable myocardial areas were stained dark red (TTC positive) (Figure 1). Slices were digitally scanned after staining and fixation at a resolution of 5506 x 3298 dpi, using a scanner (Epson Perfection model V500 PHOTO) and Adobe Photoshop Elements software. Prior to scanning, the slices were prepared by placing them between two pieces of plexiglass with a ruler. This helped prevent edges of the tissue from curling up, and provided a means for a standard measurement to calibrate the software used to measure the infarcts.

Infarct Measurement Procedure

Area measurements for each heart slice were calculated using Image Tool Software, a free shareware created by the University of Texas at San Antonio. The software allows for the initial calibration of measurement using a standard metric ruler, which was placed in each scan, followed by the calculation of the total area (TA) measurement for each heart slice once manually outlined using the software.

The total area of each slice was derived from the area measurement of the complete cross section of the heart. The Dead Space Area (DSA) was derived from the outline of any open space left in the heart slice by the atrium or ventricle. The Infarction Area (IA) was derived from the outline of the infarcted portions of each heart slice. The Total Area of Cardiac Muscle (TACM) was then derived by deducting the DSA from the TA of the heart slice (TA/slice – DSA/slice = TACM/slice). The resulting values, for each slice of cardiac tissue, were then compiled to provide the Total Area of Cardiac Muscle for the whole heart as well as the Infarcted Area (IA) of the whole heart (TACM/slice x 6 slices = TACM/heart and IA/slice x 6 slices – IA/heart). The infarction percentage was determined by dividing the heart totals for Infarcted Area (IA/heart) by the Total Area of Cardiac Muscle (TACM/heart). The consistent slice measurements of 2 mm made the use of volume for calculating infarct percentages a moot point, since it would not have changed the calculated percentages.

Results

The infarction lesion size varied from 3.12% to 30.84% of total cardiac muscle (cross section). The majority of animals (70%) had lesion sizes between 20 and 30%. As indicated, 9.6% of animals had infarction sizes smaller than 10%, over 38% of the animals had infarction sizes between 20% and 25%, and over 31% of animals had infarction sizes within 25%-30%.

Discussion

The induction of a myocardial infarction by ligation of the LAD coronary artery in the rat model is a technically challenging model, especially when trying to standardize and consistently reproduce a particular infarct size. Contributing factors include: difficulty in performing thoracic surgery in very small animals; difficulty of placing a ligature on the rapidly beating rat heart; and difficulty of coronary artery visualization and relative variability in anatomy between individuals. Surgically induced lesions in rats are classically described in the literature as small (less than 20% of total cardiac tissue), medium (20 to 40%) or large (greater than 40%). Researchers are generally interested in medium or large infarcts, as such lesions result in significant cardiac remodeling and associated physiological changes. Long-term survival after the creation of a MI is also a desirable characteristic of a pre-operated model, particularly with respect to developing and testing novel therapies. As large infarcts have a higher risk of post-operative mortality, the goal of our lab is to deliver models with medium-sized ischemic lesions. This study was designed to document the consistency of surgical outcome, and to develop guidelines for performance evaluation and certification of our surgeons.

In order to maximize time and resources, researchers often purchase experimentally induced models to use on studies. Sometimes it is desirable to screen the animals and quantify/qualify the myocardial lesion before assigning animals to specific experiments. Different *in vivo* screening tools are commercially available to the scientific community.

Echocardiography, with or without the use of Doppler and contrast agents, is a classical method which allows accurate estimation of infarct size in rodent models.[8-9] Advanced imaging modalities, such as Ventricular ejection fraction by nuclear imaging[10] (use of multiwire gamma camera), and MRI (molecular resonance imaging), or 3D MRI, are available and allow for an early evaluation of tissue damage.[11-12] Lastly, the electrocardiogram can be a useful tool for infarct verification, but it does not quantify infarct size.[13] In conclusion, the success and consistency of lesion size is highly dependent on the training received by the surgeon, skill level of the surgeon, and standardization of the surgical protocol. The results from this study indicate that a medium-sized ischemic lesion, comprising of 20-30% of total cardiac tissue, can be reliably produced in at least 70% of the animals that underwent surgery by experienced surgeons in our lab.

As such, in order for a surgeon to attain certification and provide this model commercially, he/she must be able to consistently produce medium-sized infarct lesions covering 20 to 30% of myocardial tissue, in a minimum of 70% of LAD artery ligation surgeries performed. TTC staining was a useful *in vitro* tool for assessing myocardial infarct size. It serves as a subjective indicator for validating the effectiveness of training for new surgeons, as well as a marker of success in the annual recertification of trained surgeons.

Figure 1

References

1. Panum, P.L. Experimentelle Beitrage zur Lehre von der Embolie. Virchows Arch f. path. Anat. 25: 308, 1862.
2. Fenoglio, I. and Drgoul, G. Observations sur l'occlusion des coronaries cardiaques. Arch.Ital.de boil. 9: 49, 1888.
3. Miller, J. L. and Matthews, S. A. Effects on the heart of experimental obstruction of the left coronary artery. Arch. Int. Med. 3: 476, 1909.
4. Johns TPN, Olson BJ. Experimental myocardial infarction, I: a method of coronary occlusion in small animals. Ann Surg 1954; 140:675-82.
5. Klein HH. *et al*. The mechanism of the tetrazolium reaction in identifying experimental myocardial infarction. *Virchows Arch* 1981; 393: 287-297.
6. Michael LH. *et al*. Myocardial ischemia and reperfusion: a murine model. *Am J Physiol* 1995; 269: H2147-H2154.
7. Schwarz ER *et al*. What is the required reperfusion period for assessment of myocardial infarct size using triphenyltetrazolium chloride staining in the rat? *J Thromb Thrombolysis* 2000; 10: 181-187.
8. Kanno S. *et al*. Echocardiographic evaluation of ventricular remodeling in a mouse model of myocardial infarction. *J Am Soc Echocardiogram*. 2002 Jun; 15(6):601-9.
9. Park SW. *et al*. Quantitative assessment of infarct size *in vivo* by myocardial contrast echocardiography in a murine acute myocardial infarction model. *Int J Cardiol*. 2004 Dec; 97(3):393-8).
10. Constantiensco A. *et al*. Assessment of left ventricular perfusion, volumes, and motion in mice using pinhole gated SPECT. *J Nucl Med*. 2005, Jun;46(6):1005-11.
11. Bohl S. *et al*. Advanced methods for quantification of infarct size in mice using three-dimensional, high-field late gadolinium enhancement MRI. *Am J Physiol Heart Circ Physiol*. 2009 Apr;296(4):H1200-8. Epub 2009, Feb 13.
12. Luo D. *et al*. Myocardial infarction quantification with late gadolinium-enhanced magnetic resonance imaging in rats using a 7-T scanner., *Cardiovasc Pathol*. 2011 Jun 6. [Epub ahead of print].
13. Bonilha AMM. *et al* A routine electrocardiogram should not be used to determine the size of myocardial infarction in the rat. BJMBR (2005) 38; 615-619.

09/2011