



The Sheep as a Model for Coronary Artery Surgery Experiments on Beating Heart

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Abstract

Background: A good animal model for coronary surgery experiments has been difficult to establish. An ideal model should have the closest morphological resemblance to human beings. The objective of this study is to establish sheep as a model for these experiments.

Methods: The anatomical aspects of left anterior descending coronary artery (LAD) and right internal thoracic artery (RITA) in the sheep are studied. Coronary artery bypass grafting between the RITA and LAD coronary artery was performed. Patency of the anastomosis was evaluated by follow-up angiography.

On a beating heart, the RITA was anastomosed to the LAD in adult sheep. A left anterior thoracotomy in the fifth intercostals space gave good access to both vessels. Ventricular fibrillation (VF) was a major intra-operative problem. Its incidence and relation to ischemic time was studied. The anastomosis patency was tested immediately and at follow-up by a modified technique of angiography. The morphological anatomy of both LAD and RITA was studied in detail and analysed. Surviving sheep were studied for 6 months or more.

Results: RITA was easy to harvest. The most common anatomy for the LAD was presence of two diagonal branches and absence of an overlying vein. The incidence of ventricular fibrillation (VF) during LAD snaring was 10.8% (mean ischemic time before VF occurrence was 4 minutes). The modified angiography technique produced good quality angiograms. Wound infection was initially a problem but controlled with prophylactic antibiotics.

Conclusion: Favorability of RITA and LAD anatomy prove sheep as a good animal model for coronary artery surgery experiments. VF incidence is acceptable. Wound infection is controlled. Good quality follow-up is feasible.

The Journal of Tehran Heart Center 1 (2006) 11-15

KeyWords: Sheep • Model • Coronary artery surgery

Introduction

Coronary artery bypass surgery has been performed over the last 30 years in large numbers: Now the number of operations in the western world is closer to one million

per year. Despite the generally excellent results with this technique, there remains some associated mortality and morbidity particularly in patients with increased risk factors.^{1,2} Hence, over the last decade, there has been an intensive focus on less invasive surgery leading to the

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development of minimally invasive and off pump coronary surgery.^{3,4,5} To facilitate this, new approaches and instruments were developed. New techniques and technologies have to be tested on animal models before implementation in humans. A good animal model for coronary artery surgery has been difficult to establish. An ideal model should have the closest morphological resemblance to human being.

In spite of the fact that they are stressful animals, pigs have been used because of their heart's similarities to human heart, including a small capacity for coronary collateral flow.⁶ The weight of dogs and the size of the coronary arteries are limiting factors for their use. Until now, nobody has reported on beating heart surgery with long term follow up in a sheep model. In our animal laboratory a new anastomotic device was tested in sheep. Data about the anastomotic device were published earlier.⁷ This paper aims to analyze the anatomical aspect of the left anterior descending coronary artery and the right internal thoracic artery in the sheep describing this experimental animal model in detail.

Methods

Animals

Coronary artery bypass between the right internal thoracic artery (RITA) and the left anterior descending artery (LAD), either by using an anastomotic device or by continuous 7-0 Polypropylene sutures, was carried out at our institution's animal laboratory in 65 sheep. These were healthy sheep of the local Najdi and Naimi breeds, 8-12 months in age, corresponding to a body weight of 40-55 kg, chosen and obtained from the local market.

They were taken to the Animal Care Facility of the, Research Centre, underwent physical Examination and routine laboratory workup, then given prophylactic penicillin or cephalosporin. After a period of at least two weeks and when their health had been determined to be satisfactory, they were used for experiments. The experimental protocol was approved by our Research Advisory Council and Animal Care and Use Committee. The animals were fasted for 24-36 hours with access to water until 8 hours prior to surgery. Before the procedure, 1 gram of intravenous cefazolin sodium (Keflin; Eli Lilly; Indiana, USA) was given followed by Gentamycin (Gentamycin; American Pharmaceutical, Illinois, USA) 1gm/day for 5 days.

Surgical procedure

A central venous line was obtained by puncturing the external jugular vein. The right carotid artery was openly accessed and cannulated for monitoring the arterial pressure and obtaining arterial blood gas samples. The arterial blood gases were monitored every 30 minutes. Atropine sulphate (Atropisol; American Regent; New York) 0.2 mg/kg, was

given preoperatively and every 15-30 minutes during the procedure. For muscle relaxation, atracurium besylate (Tracrium; Calmic, UK or; Wellcome, USA), 0.2 mg/kg was given and for reversal neostigmine (Prostigmin; Roche; UK), 0.5-2.5 mg. Propofol (Diprivan; ICI Pharmaceuticals; UK), 4 mg/kg was used for induction and maintenance as required. Halothane (Fluothane; ICI Pharmaceuticals; UK), 0.8-1.5 vol. % was added after induction of anaesthesia. Extremity EKG and oximetry with a tongue probe were used for additional monitoring.

The sheep sternum was found too thick for a median sternotomy; it was difficult to split and achieve a stable closure. The internal thoracic artery was found to be located in the angle formed between the sternum and the ribs. A left thoracotomy in the 5th intercostal space was the approach of choice. It was extended half way across the sternum at the beginning of our experience. It allowed a good exposure of the heart and the right internal thoracic artery the pericardial cavity was found to be suspended by two ligaments-one to the diaphragm, and the other to the sternum.

When these ligaments had been split, the heart and the pericardium were mobile and the RITA was well exposed through an anterior opening in the right pleura. It was worth mentioning that an ample space was found retrosternally. The left internal thoracic artery was ligated and transected.

The RITA was inspected and then harvested without pedicle. The morphological anatomy, the side branches and the size of the RITA were studied. Thereafter, 100 units/kg of heparin sodium (Unihep; Leo; United Kingdom) and 1 mg/kg/min. of Lidocaine (Lidocaine HCL injection; USP; USA) were given intravenously as a bolus, followed by 0.1 mg/kg/min of Lidocaine (Lidocaine HCL injection; USP; USA), Propranolol (Inderal; ICI Pharmaceuticals; United Kingdom), 1.6mg or esmolol (Brevibloc; DuPont; USA), 0.5-1 mg was given, as needed, to achieve a heart rate below 80 bpm, since the risk of ventricular fibrillation (VF) increases with higher heart rate.⁸ Five ml of Xylocaine 2% (Xylocaine; Astra; United Kingdom) was applied topically to the epicardium and instilled in the pericardial cavity. The LAD was explored. The number of diagonal branches and the presence or absence of overlying veins was noted. A 4-0 Polypropylene suture for snaring was placed around the proximal LAD. On the beating heart and without a stabilizer, the LAD was opened for approximately 6 mm. The LAD was snared proximal to the anastomotic site. Preferably, location for the snare was chosen distal to the first diagonal branch of the LAD to minimize the amount of ischemic muscle and the risk of VF. The size of the LAD was measured with coronary probes. The ischemic time before the appearance of any VF was recorded. In many instances, the LAD was located behind the anterior cardiac vein, which resulted in bleeding when the LAD had been dissected free. The RITA was anastomosed to the LAD between the first and the second diagonal or distal to the second diagonal branch. After the release of the snare, the blood flow in the RITA was measured



by a transit time flow meter (Transonic Systems, Inc. Ithaca, NY) usually using a 2.5 mm probe. Thereafter, the LAD was ligated proximal to the first diagonal branch. Two flows were measured - one, with the proximal LAD open, and the other with the proximal LAD closed. The survival criteria were: open anastomosis on fluoroscopy, detected RITA flow and stable hemodynamics at the end of the experiment.

In 3 sheep, a length of 10-15 mm of the LAD was dissected free from the surrounding tissue one centimetre proximal to the first diagonal and the LAD flow was measured using the same flow probe.

Fluoroscopy and follow up

The patency of the anastomosis performed between the RITA and the LAD was tested immediately. Contrast was injected through a 24-gauge arterial cannula (Omnipaque, Nycomed, Oslo, Norway) inserted into RITA and connected to an extension line. Later, the patency was checked 1 month, 3 months and 6 months postoperatively. The technique for repeat angiography is as follows: the RITA was approached directly via an incision parallel to the right sternocleidomastoid muscle on the neck. The sternocleidomastoid was transected and the right subclavian artery (RSA) dissected free. The cephalic and the subclavian veins that interiorly cross the former artery were divided allowing a good mobilization of the RSA. The origin of the RITA was found to be behind the confluence of the right subclavian vein and the right jugular vein. Two to four angiographies were performed in the same animal and through the same incision with increasing amount of adhesions (Figure 1).

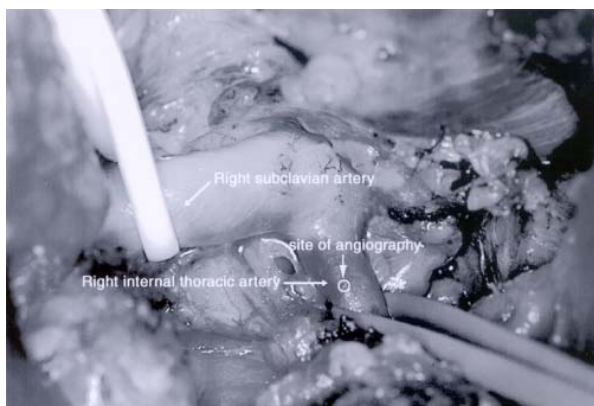


Figure 1. Site of angiography needle puncture on the right internal thoracic artery. Note the right subclavian artery is surrounded with a loop

All animals received humane care in compliance with the European Convention on Animal Care.

Results

Sixty-five sheep were analyzed for this publication. They

had their anastomosis performed either by suturing or with a new anastomotic device.

Anatomy

The RITA was clearly identified approximately 2 cm from the right sternal border, lying on the chondral segments of the ribs and was accompanied by two veins. Two segments of the RITA were defined. The proximal part (40%) was covered by the parietal pleura and was separated from the chest wall by dense tissue. In this segment, there were 4 to 6 side branches, the dissection of which was easy. This distal segment was hidden by muscles, rich with side branches and was much more difficult to harvest. Small metal clips were used for controlling the side branches. The quality of the RITA was analysed on the basis of 3 elements: the diameter (more than 2.5 mm or less than 2 mm), the presence or the absence of luminal dissection and the length. The anatomical description of the RITA was available in 59 cases. The RITA was poor in two cases because of luminal dissection in one sheep and because it was short in the other. The LAD was generally good but was poor in 7 cases because of small size (less than 1.5 mm). The most common anatomy was the presence of two diagonal branches. The number of diagonals and the presence of overlying veins are summarized in (Table 1).

Table 1. Characteristics of the LAD

| Size (n=58) | LAD | |
|---------------------|---------------------|----------------------------|
| | Diagonals (n=55) | Over lying veins (n=55) |
| >1.5 mm 7(12%) | One 3 (5%) | Yes 18 (33%) |
| 1.5 – 2 mm 45 (78%) | Two 49 (89%) | No 37 (67%) |
| >2mm 6 (10%) | Three 2 (4%) | |
| | Four 1 (2%) | |

RITA blood measurement

The blood flow was measured in the RITA peroperatively with and without closure of the proximal LAD. It varied from 0 to 34 ml/min when the proximal LAD was open (mean of 8.4 ml/min) and 7 to 58 ml/min (mean of 23.9 ml/min) when the proximal LAD was closed. The native flow measured in the LAD of three animals was 15, 15 and 18 ml/min, respectively.

Fluoroscopy and angiography

A total of 96 angiographies were done during this study. It was worth mentioning that at the beginning of the experiments we did not manage to catheterize the RITA via femoral, axillary, brachial or radial access, hence, we abandoned this approach. The quality of the images improved a lot when

we succeeded to catheterize the RITA directly as described above (Figure 1).

Mortality and morbidity

Twelve (18.5%) animals expired during the surgery. Three were sacrificed because they did not meet the survival criteria. The other 9 had ventricular fibrillation (VF) and died during surgery. The VF occurred during the snaring of the LAD in 7 cases and mainly during the performance of the first 33 anastomosis. The mean ischemic time before the occurrence of VF was 4 minutes ranging from 1.5 to 8 minutes? As our experience improved, we learned to avoid VF which occurred only in 2 cases during the second half of the study. Fifty-three sheep survived the surgery. Eight among them had chest wound infections (15%). In seven of these (87.5%), the wound infection complicated the extended thoracotomies to the sternum. Sixteen sheep died within 30 days following the procedure. The causes are summarized in (Table 2).

Table 2. Causes of late death (n = 16)

| Causes | Number |
|--------------------|--------|
| Bleeding | 2 |
| VF due to contrast | 3 |
| Chest Infections | 4 |
| Endocarditis | 1 |
| Arrhythmia | 1 |
| Heat Stroke | 2 |
| AMI | 1 |
| Undetermined | 2 |

VF, Ventricular Fibrillation; AMI, Acute Myocardial Infarction

Thirty-seven animals were sacrificed according to the protocol, 4 had minor chest infection which did not compromise the quality of the angiographies.

Discussion

The sheep as an animal model has been used previously for cardiac valve surgery.⁹ the sheep grows slowly is docile and its coagulation system is similar to that of humans.¹⁰ although there has been an increasing interest for beating heart and minimally invasive surgery, the sheep was rarely used for coronary surgery experiments.

In order to test a new anastomotic device, 65 sheep underwent coronary artery procedures on the left anterior descending artery on a beating heart. It was easy to approach the whole LAD through the fifth left intercostal space. David J. Farrar used the fourth intercostal space for implanting a prosthetic coronary artery bypass graft on the proximal

circumflex in a sheep model as well.¹¹ the exposure was good without respecting any rib. At the beginning of our experience, we used to extend the incision half way across the sternum when the animal was fat. We later abandoned this extension, as we experienced recurrent and fatal chest wound infections in the extended incision animals. Actually, the sheep is an animal that rests on the sternum, which exposes the wound to fecal contamination of the floor. To prevent this complication, we adopted strict aseptic measures when dealing with sheep during anaesthesia and surgery, reduced the size of skin incisions and dressed the wound for 48 hours. In addition, prophylactic antibiotics were used for 5 days. This led to reduction in the wound infection rate. The administration of prophylactic antibiotics, after implantation of valve prosthesis until the wound became dry and healed, was used in dogs and sheep.¹²

The origin of the RITA is in the concavity of the right subclavian artery and at approximately 2 cm from the sternal border. It is accompanied by a pair of internal mammary veins. The RITA is covered by the parietal pleura only in its proximal one third; below this level, muscles separate the vessel from the pleura. In contrast to human anatomy, the majority and the largest side branches are located at this distal part of the RITA. We found it easier to harvest a skeletonized RITA rather than on a pedicle. All the branches were secured with metal clips and cut in order to have a good mobilization, though it has been proven recently in pig models that patent side branches do not affect coronary blood flow in internal thoracic artery to left anterior descending anastomosis.¹³ a good length of the RITA proved to be important. In the follow up angiograms we found the distal RITA stenosis to be related to stretch conduits. In our study, the LAD of the sheep had two diagonals in 90% of cases. The anastomosis of RITA to LAD was generally done distal to the second diagonal. When the LAD was covered by a vein or was intra-mural, its dissection became difficult and bleeding from damaged veins was common. During the anastomosis, the LAD was snared distal to the first diagonal. This gave us enough time to perform the anastomosis, as most of the myocardium was still perfused by the first diagonal. While completing the anastomosis, we experienced many cases of ventricular fibrillation (10.8%), mainly when there was only one diagonal or when the second diagonal was dominant. The occurrence of VF decreased significantly as we became experienced to perform anastomosis with the new device. The incidence of VF after snaring of the proximal LAD in pigs has been reported to be between 71% and 100%.¹⁴ This difference in the VF occurrence when the LAD was snared inferred that the LAD of the sheep has more collaterals than that of the pig. The dog is less prone to VF after the occlusion of the proximal LAD because the circumflex supplies more myocardium than does the LAD in pigs.¹⁴ Moreover the dog has a rich capacity of collateral flow. The occurrence of arrhythmia after ligation of a coronary artery probably depends on the amount of collateral flow. In pilot tests, we



did acute experiments on dogs and were happy with the absence of VF but we were not satisfied with small size of the LAD.

When the anastomosis had been done and the flow had been measured, the LAD was ligated proximal to the first diagonal in order to avoid the competitive flow to the RITA from the proximal LAD.

At follow up, we recanalization of the ligated LAD, evident at angiography or at sacrifice. This problem was met even after we had changed the ligature used from polypropylene to silk. Because of this problem, in our last animals, we dissected and clipped the LAD at the origin. The elimination of the competitive blood coming from the proximal LAD allowed an undisturbed flow from the RITA.

In total, we performed 96 angiographies during the follow up. Early on we realized that it was very difficult to cannulate the RITA from a femoral, axillary, brachial or radial approach. Therefore, the approach was modified. The RITA was dissected and catheterized directly at its origin via an incision in the neck. Up to three angiographies were done using this same approach. During the third and fourth controls we encountered a lot of adhesions and experienced serious bleeding from around the superior vena cava and the right common carotid artery but never compromised the quality of the angiograms. In summary we present here the sheep as an animal model for coronary artery surgery. Its size is ideal; the coronary anatomy is suitable and is an easy animal to care for. Ventricular fibrillation is acceptable and is related to length of ischemia. Use of prophylactic antibiotics is recommended and should reduce the rate of wound infection. The retrosternal space is adequate enough to allow using the sheep for evaluating the newly developed endoscopic surgical techniques in cardiac surgery as interest in these is gaining a lot of momentum.

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