The Development of the Yucatan Miniature Pig as a Chronic Model for Cardiac Surgery

Roger A. Vertrees, BA, CCP, Joseph Costabile, MD, Linda Collins, CRNA, Paul J. Kerins, BS, Yan Yu, MD, Jonathan H. Cilley Jr., MD, William A. Baldino, MD, Anthony J. DelRossi, MD

The Cooper Hospital/ University Medical Center
School of Cardiovascular Perfusion
University of Medicine and Dentistry of New Jersey
Robert Wood Johnson Medical School
Camden, NJ

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ABSTRACT

For purposes of developing a chronic model for coronary artery bypass grafting (CABG), the Yucatan miniature pig was selected. Its cardiac and vascular anatomical similiarities to humans could be useful in achieving a model which would allow for investigations in vivo. Many difficulties have been encountered, resulting in one long-term survivor in the first series of nine animals. Of these nine pigs, there have been operative deaths due to friable venous tissue, fatal arrhythmia and exsanguination. Four others died in the post-operative period due to problems such as fatal arrhythmias, pneumothorax and hypoxia. Improved techniques in the anesthetic and surgical protocols, autologous recovery of the animals' lost blood, and the use of supplemental oxygen post-operatively have helped to overcome these problems. The next two animals survived well into the post-operative period. The last series of five Yucatan swine were all electively euthanized after they had recovered from their protocol. It is believed that the Yucatan swine, even though problems exist, may be an acceptable model for use in cardiac surgical procedures requiring a recovery or chronic phase.

INTRODUCTION

In order to evaluate the effect of certain cardioplegia additives, a chronic survivor of coronary artery bypass grafting was needed. It was also felt that a model of this kind would have many other applications as well. The dog, having been the animal of choice for so many years, is losing ground in acceptance for a number of scientific and social reasons.[1] The myocardium of the dog is richly supplied with collateral blood flow making predictable ischemia and infarction virtually impossible. Less outside attention is generated when non-companion animals such as the pig or sheep are used as opposed to the canine species. At this time, the sheep was eliminated on the basis of it being a ruminant and the special problems that are associated with this type of digestive system. Also, the sheep anatomically presents a problem in aortic occlusion due to a bracirocephalic trunk that arises from the ascending aorta. So the swine was selected.[2] In selecting a breed of swine to use, a couple of salient points were considered. The animal must be small enough to handle safely and at the same time have reached maturity or, at least, that the heart size not grow an appreciable amount.[2] On this basis one of the miniature swine breeds was selected. The Yucatan miniature swine reportedly being resistant to malignant hyperthermia and readily available in our area was then selected. The Yucatan swine is specifically bred for research purposes and because of this is an expensive animal at $300 each.[a]

Address correspondence to: Roger A. Vertrees, BA, CCP, The Cooper Hospital/University Medical Center, School of Cardiovascular Perfusion, 310 Sarah Cooper Building, Camden, NJ 08103

MATERIALS AND METHODS

All animals were operated on under sterile conditions in accordance with the "Guide for the Care and Use of Laboratory Animals."[4] Yucatan miniature pigs with an average weight of 35.5 (± 12.6 kg.) were anesthesized using ketamine, 800 mg. and acepromazine, 8 mg. intravenously (I.V.). Anesthesia was maintained with sodium pentobarbital given as required. After an initial stabilization period for anesthesia, the animal was positioned supine upon the OR table and secured by restraining the limbs. Endotracheal intubation was carried out using a long 50 cm. laryngoscope blade,[b] and was verified by auscultation of breath sounds. A four lead electrocardiogram (EKG) was applied to the extremities using a skin electrode. The EKG was monitored throughout the procedure.The sternum, bilateral groins, and the area between the cicothyroid cartilage and the suprasternal notch were surgically prepared using a betadine paint and barrier draping. The left groin was entered and dissection and isolation of the femoral artery and vein was accomplished. The left femoral artery was cannulated with an 18 gauge catheter for purposes of arterial blood pressure monitoring. This catheter was connected to the appropriate pressure transducer. The left femoral vein was cannulated using a 16 gauge I.V. needle for the purpose of having a large bore I.V. readily available. Initially the vein was harvested from the right

a. Parco/West Jersey, Winona, NJ
b. Central, Inc. Veterinary Supply, Westminster, MA

femoral vein, but it proved to be inadequate for animals one and two. A much better bypass conduit was located in the neck, the external jugular vein (EJV). This vein was used in the rest of the procedure and worked quite successfully. An added benefit of harvesting of the EJV was that the remaining stump was cannulated and venous pressures were monitored. The sternum was opened in much the same way as in the human. A notable exception is in the area of the manubrium, the bone becomes quite thick and the saw proved to be incapable of dividing it. Immediately posterior to the bony area is a very large inominate vein so care must be exercised when using sharp instruments in order to avoid lacerating this vein. Once the sternum has been retracted, it's incredible how much similiarity exists between the human and swine cardiovascular anatomy. The aorta was dissected away from the pulmonary artery and the adventitia was removed only on the anterior surface and only in the area of the proximal anastomosis. Two purse string sutures were applied to the anterior surface of the aorta where arterial cannulation would take place. This arterial cannulation site must be below the pericardial reflection and must have the adventitia left in place. The right atrial appendage was encircled with purse string suture in anticipation of cannulation. Since the swine is quite prone to arrhythmias, care must be exercised in this maneuver by staying in the area of the appendage. Aortic cannulation was carried out much like it is in humans and then connected to the arterial line of the heart-lung machine. The venous drainage catheter, a single stage, is inserted into the right atrium and secured there. The swine have a hemiazygos vein that drains into the right atrium, so drainage of this substantial blood volume must be allowed for **(Figure 1).**

An asanguinous crystalloid prime consisting of Ringer's Injection[c], 250 units of heparin, and one ampule of 50 mEq. of sodium bicarbonate were used to prime the extra-corporeal circuit (ECC). A COBE VPCML[d] oxygenator was used in conjunction with a Sarns[e] heart-lung machine. Preparation of the bypass apparatus did not substantially differ from the routine human procedure. The animal was anticoagulated with heparin, 2.5 mg/kg./bw, given three minutes prior to cannulation. Blood flows on bypass ranged from a low of 30 cc/kg/min. at hypothermia and to a high of 85 cc/kg/min. Adequate oxygenation and tissue perfusion were manifested throughout the procedures.

After the aortic occlusion clamp was applied, the heart was arrested using a 4:1, blood: crystalloid solution containing 48 mEq/KCL, 350 cc Ringer's, 31.25 grams of mannitol, 11 mEq. $NaHCO_3$, and 250 mg. Lidocaine. The cardioplegic solution was administered into the aortic root through a 16 gauge needle for three minutes at a perfusion pressure of 300 Torr using the Sarns MP-4 system.[f] The left anterior descending coronary artery (LAD) was located and prepared for grafting. The outflow or distal end of the EJV was anastomosed to the LAD and the proximal end or inflow end was anastomosed to the aorta. The use of a left ventricular decompression sump is indicated should the surgical field fill with blood from the coronary artery.

After the distal anastamosis was completed, the proximal rewarming of the subject by the heart-lung machine is started. Rewarming from 28°C rectal temperature to about 34°C rectal averaged 28.4 minutes. Once the animal is thoroughly warmed, bypass is discontinued and the anticoagulation reversed with protamine sulfate in the ratio of 1.1 mg. of protamine to 1.0 mg. of heparin. Decannulation is completed and surgical hemostasis is ensured. The operative sites were closed following accepted protocols. Once surgery had been completed, the animal was turned over onto its left side and is allowed to awaken.

DISCUSSION

The Yucatan miniature pig was selected for this protocol due to its size limitations, known cardiac anatomy, response to cardioplegic solutions and known physiological parameters.[8] **Table 1** lists the results of an arterial blood sampled for gases, electrolytes and hemoglobin and **Table 2** displays the results of the coagulation screen done on these animals.As can be seen in **Table 3**, animals 1 and 2 died from exsanguination, or uncontrolled hemmorhage during the operative procedure. There were 3 operative deaths, the other being animal 9. In animal 1, the femoral vein was utilized as the bypass conduit and proved to be inadequate. The swine having short legs and not being a running, exercising animal has basically inadequate leg veins. Not only is the vein too short to reach from the LAD to the aorta, but the consistency is such that when an arterialized pressure was perfusing through the vein, the excess pressure caused bleeding through the wall of the vein to occur resulting in uncontrolled hemmorhage. In animal 2, aortic cannulation was carried out in an area above the pericardial reflection in which the adventitia had been removed and control of bleeding in this area was never realized.[1] Bleeding is a problem in swine, the venous tissue is friable and as such requires the use of buttress sutures and pledgetts. After dealing with the bleeding problem, the next series of deaths occurred early in the post-operative period. Animals 4 and 5 both succumbed due to hypoxia. Animal 4 had the endotracheal tube placed only into the larynx and consequently post-operatively started a gastric distension which caused a further problem with the positioning until the animal died from hypoxia. Animal 5 was also a victim of the foregoing analog. Upon the demise of this animal a necropsy was performed in which it was realized that the human endotracheal tube was insufficient in length. **Figure 2** is an artist's description of the pulmonary anatomy of this pig. A redesigned tube of 33 cm. was used in the rest of the animals eliminating this as a problem. This tube is pictured in **Figure 3**. After the death of animal 6 from a fatal arrhythmia, 1 gm/250cc D5W of Lidocaine was administered from the start of surgery through to its completion. This prophylactic measure worked well in all the rest of the animals except 9. Indeed, an I.V. administration of a prophylactic dosage of Bretlyium was also necessary to help quiet the ventricular arrhythmias. It is documented that swine do suffer from arrhythmias as a result of cardiac surgery and our

c Travenol Laboratories, Deerfield, IL
d COBE Laboratories, Lakewood, CO
e, f SARNS/3M, Ann Arbor, MI

work seems to support this.[6] Animals 7 and 8 succumbed from a confusing series of events culminating in right heart failure seemingly due to pulmonary hypertension. Possible causes of this could be particulate matter from the perfusion or what is more probable is "micro-atelectasis" or hypo-alveolar ventilation. This disarrangement results in a ventilation, perfusion mismatch wherein oxygen cannot get to the blood. Some causes could be a prolonged dorsal position and lack of the "sigh" reflex. Two things that may help in dealing with this phenomenon are the addition of positive end expiratory pressure (PEEP) and a post-operative position of sternal recumbency. In the next protocol, six miniature Yucatan swine were subjected to heparinless bypass and using the aforementioned solutions. All the animals in this protocol lived to be electively euthanized post-operatively.[5]

CONCLUSION

In attempting to develop the Yucatan miniature swine to serve as a chronic model for CABG procedures, many problems were encountered. If the animal died during surgery it was probably due to hemorrhage as seen in Graph 1. Should the subject die within an hour of completion of surgery, then hypoxia is the cause. If the subject expires within two hours post-operatively, then our problem is cardiac arrhythmia. Should all these problems be effectively dealt with, then the pulmonary hypertension that has proved fatal after about 12 hours must be dealt with.[6] By using reinforced sutures on the venous structures, tying sutures without pressure on the suture line, using adventitia on arterial structures, and staying below the pericardial reflection, (**Figure 3**) the problem of bleeding was effectively dealt with. It was found to be necessary to utilize the EJV for the arterial conduit as opposed to the femoral vein. A specially designed endotracheal tube, 33 cm. in length, was found to be necessary in order to adequately ventilate the swine. The arrhythmias were eliminated by infusion of a prophylactic dosage of Lidocaine and Bretylium and in the next protocol, the hypoalveolar ventilation was combatted by adding 5 cm. PEEP to the system (Graph 2 summarizes these experiences). The swine as a model for chronic CABG surgery is wrought with many problems. That the swine's heart is no stranger to cardiac surgery can be seen in many authors work in defining the composition of cardioplegic solutions. This was one of the primary reasons for utilizing this animal in attempting to define a chronic model for CABG. In light of the fact of persistent arrythmias and the hypoalveolar ventilation, it may be prudent to investigate another species.

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REFERENCES

1. Panepinto LM, Philips RW.: The Yucatan miniature pig: characterization and utilization in biomedical research. *Laboratory Animal Science*, Vol. 36, No. 4, pp. 344-347, 1986.

2. Swindle MM, Smith AC, Hepburn BJS.: Swine as models in experimental surgery. *J. of Invest. Surg.*, Vol. 1, pp. 65-79, 1988.

3. Swindle MM, Horneffer PJ, Gardner TJ, et.al.: Anatomy and anesthetic considerations in experimental cardiopulmonary surgery in swine. *Laboratory Animal Science*, Vol. 36, No. 4, pp. 357-361, 1986.

4. National Academy of Sciences: Guide for the Care and Use of Laboratory Animals, NIH Publication, No. 80-23, Rev. 1978.

5. DelRossi AJ, Cernaianu A, Vertrees RA, et.al.: Heparinless extra-corporeal bypass for treatment of hypothermia. Submitted, *J. of Thorac. and Cardiovasc. Surg.*, 1989.

6. Swine in cardiovascular research, Laboratory Animal Science, Copyright 1986, *Amer. Ass. for Lab. An. Science*, Vol. 36, No.4, 1986.

7. Horneffer PJ, Gott VL, Gardner TJ.: Swine as a cardiac surgical model. *Ann. Thorac. Surg.*, 40:574-581, 1985.

8. Radin MJ, Weiser MG, Fettman MJ.: Hematologic and serum biochemical values for Yucatan miniature swine. Laboratory Animal Science, *Amer. Ass. for Lab. An. Science*, Vol. 36, No. 4, Copyright, 1986.

TABLE #1

ARTERIAL BLOOD ANALYSIS

TEST	MEAN	RANGE	UNITS
GLUCOSE	80	56.0-150.0	mg/dl
SODIUM	147.0	142.0-153.0	mEq/l
POTASSIUM	4.6	3.9-5.2	mEq/l
CHLORIDE	104.2	95.0-114.0	mEq/l
CALCIUM	10.6	9.3-11.5	mg/dl
HEMAGLOBIN	15.0	13.0-17.0	g/dl
HEMATOCRIT	44.6	36.0-53.0	0/0
PO2	90	85-105	Torr
PCO2	40	35-44	Torr
pH	7.39	7.35-7.44	
BLOOD PRESSURE	110/60 $\bar{m}$=85		mm/Hg
CVP	4-6		mm/Hg

TABLE #2

COAGULATION PANEL

TEST	RESULTS
BLEEDING TIME	1-5 (min)
LEE-WHITE CLOTTING TIME	1-5 (min)
ACTIVATED CLOTTING TIME	105 (sec)
PROTHROMBIN TIME	9-14 (sec)
PARTIAL PROTHOMBIN TIME	<38 (sec)
PLATELET COUNT	12-72 x 10^4 /ml or 120-720 x 10^3 /dl
FIBRINOGEN	131 mg/dl
FSP	<10 mg

TABLE #3

CAUSE OF DEATH

#	SEX	WT (KG)	TIME OF DEATH	CAUSE OF DEATH
1	F	59.6	OR	VEIN GRAFT, INADEQ. EXSANGUINATION
2	M	28.9	OR	INADEQUATE EXSANGUINATION
3	M	38	60 DAYS POST-OP	EUTHANASIA
4	M	34.4	1 HR. POST-OP	HYPOXIA
5	M	55.6	1 HR. POST-OP	HYPOXIA
6	M	59	2 HR. POST-OP	ARRHYTHMIA
7.	M	31	10 HR. POST-OP	RIGHT HEART FAILURE
8.	F	36	12 HR. POST-OP	RIGHT HEART FAILURE
9.	M	26	OR	ARRHYTHMIA
10.	M	31	24 HR. POST-OP	EUTHANASIA
11.	F	25	4 HR. POST-OP	EUTHANASIA
12.	M	25	14 DAYS POST-OP	EUTHANASIA
13.	M	23	4 HR. POST-OP	EUTHANASIA
14.	M	24	12 HR. POST-OP	EUTHANASIA