

Evaluation of the feasibility of an experimental model of left ventricular hypotrophy and retraining

ABSTRACT

Fundamental Premise: There are clinical situations in which the unprepared left ventricle (LV) cannot maintain normal cardiac output.

Objective: To evaluate the technical feasibility of the experimental model to the unprepared LV which will reproduce the anatomical and functional conditions of hypotrophy, considering abdominal heterotopic cardiac transplantation; to evaluate the survival of transplanted animals and morphological and morphometric cardiac variations.

Material and Methods: During a 10-month period, 143 abdominal heterotopic cardiac transplants were performed using the modified Ono-Lindsay technique, using male Sprague-Dawley rats, with weights mean 294.4 ± 21 g. The longer and shorter axes and ventricular masses were measured in transplanted and native hearts.

Results: Eight rats lived longer than 12 hours postoperatively. Five of these lived a mean of 125.6 ± 35.8 days, 67 rats lived up to 12 hours: mean 10 ± 6 hours, and 68 rats died in the first 30 minutes postoperatively: mean 24 ± 14 min. The main causes of failure were bleeding, detachment of fibrinoid emboli with later impact on the coronary ostia and poor myocardial protection. In the anatomopathological study of 5 rats, the longer axis in the native hearts and the transplanted ones were an average of 1.46 ± 0.26 cm and 1.12 ± 0.22 cm, respectively, $P=0.04$; shorter axis: 1.56 ± 1.27 cm and 1.36 ± 0.48 cm, respectively, $P=0.14$; the ventricular mass in the native hearts and the transplanted ones were, respectively, $1,260 \pm 0.16$ g and $1,013 \pm 0.27$ g, $P=0.04$.

Conclusion: The experimental model in rats, to evaluate hypotrophy of the LV is technically feasible, although it requires an extensive phase of training. The anatomopathological measures showed hypotrophy of the transplanted hearts.

KEY WORDS: Models, Animal – Heart Ventricle – Heart Transplantation.

GABRIEL LORIER – Doutor em Cardiologia pela FUC. Cirurgião cardíaco da Asociación Española Primera de Socorros Mutuos. Montevideo – Uruguai.

RENATO A.K. KALIL – Doutor em Cardiologia pela UFRGS. Cirurgião cardíaco do Instituto de Cardiologia do RS.

Instituto de Cardiologia do Rio Grande do Sul. Fundação Universitária de Cardiologia.

✉ Endereço para correspondência:

Dr. Renato A.K. Kalil

Av. Princesa Isabel, 370

90620-001 – Porto Alegre – RS – Brasil

00-55-51-3230.3600 Ext.3777

✉ pesquisa@cardnet.tche.br

and morphometric study of the transplanted and native hearts.

MATERIAL AND METHODS

The present study was performed in the Laboratory of Experimental Surgery at the Marie Lannelongue Surgical Center (CCML – Centre de Chirurgie Marie Lannelongue), connected to Université Paris-Sud at Le Plessis Robinson, France. In a 10-month period, 143 abdominal heterotopic cardiac transplantations were performed using the modified Ono– Lindsay technique (4).

This is an experimental model of abdominal heterotopic cardiac transplantation, whose vascular anastomoses must be performed according to microsurgical techniques. For this purpose a Zeiss Op-Mi6 microsurgical microscope (focus=16/125, Carl Zeiss, West Germany) was used, besides instruments compatible with microsurgery.

The donor and recipient hearts were weighed on a BP210S precision scale (Sartorius AG, Göttingen, Germany).

Two hundred and eighty-six male rats (*ratus norvegicus*) of the Sprague-Dawley race (Harlan France, Gannat, France), were used, with weights ranging from 280 to 350 g (mean: $296,4 \pm 21$ g).

The animals were treated according to law (Decree 87 – 848, applying articles 454 of Criminal Law and 276 of Rural Law, Republic of France).

INTRODUCTION

There is a population of children with transposition of the great vessels (TGV), and an intact interventricular septum, which present with a left ventricular mass of less than 30 g/m^2 body surface, or left ventricular LV pressure less than 50mmHg. These left ventricles do not have a structural or functional capacity to support a systemic load (1). In such patients, a period of LV retraining will be necessary to perform an anatomical correction using Jatene's surgical procedure.

In literature, we do not know an experimental model that will reproduce the anatomical and functional conditions, so that a same LV can run sequentially in time through the phases of unpreparedness and retraining as physiologically as possible. The creation of an experimental model for

this purpose would allow one to characterize the LV retraining phase from the anatomical, physiological, histological, metabolic and genetic standpoints, as well as to perform pre-clinical tests that will enable the continued optimization of the surgical LV retraining method as proposed by Yacoub in 1977 (2,3). New associated therapeutic methods could also be tested, such as angiogenic therapy. In this study we report our experience in creating an experimental model that would enable one to study the molecular bases of physiological retraining of the unprepared LV.

Our objectives were to evaluate the technical feasibility of the experimental model of retraining of the unprepared left ventricle, by means of abdominal heterotopic cardiac transplantation in rats, and to perform an anatomopathological, morphological

The rats were anesthetized with 0.5 ml of anesthetic solution intraperitoneally (ip). The composition of each ml of anesthetic solution is:

- a) 0.8 ml of ketamine (Kétalar®, Parke Davies, Courbevoie, France);
- b) 0.2 ml of xylazine (Rompun®, Bayer Pharma, Puteaux, France).

The solution was applied again as needed (0.2 ml ip).

Preparing the Recipient:

- a) xiphopubic median laparotomy associated bilaterally with two sub-costal incisions;
- b) careful dissection and ligation of lumbar veins using propylene 7-0 (Prolene®, Ethicon®, Johnson & Jonson);
- c) the abdominal aorta and inferior vena cava were kept contiguous, without dissecting the intervacular plane, since: (i) there is a fragilization of the vena cava wall, causing bleeding after vascular occluding are released; (ii) there is an unnecessary increase in surgical time; (iii) the contiguosness of the vessels does not interfere with performing the anastomoses;
- d) positioning of the vascular tourniquets in a proximal position to the emergence of the ileolumbar veins, and distal right above the emergence of the iliac arteries (the tourniquet was 5 cm long and was assembled with: polypropylene 5-0 Prolene®, Ethicon®, Johnson & Johnson, Brussels, Belgium); and an 18G intravenous catheter (Vygon, Ecouen, France);

Preparing the Donor:

After immobilizing the animal in dorsal decubitus, initially a cervical approach was used to access the airway, followed by an abdominal approach.

The abdominal part was as follows:

With the animal in dorsal decubitus, the abdominal approach is identical to that used for the recipient. After dissection, the inferior vena cava was punctured with a 24 G needle (Ethicon®, Johnson & Johnson Medical, Pomezia, Italy) and sodium heparine was injected (Heparine Sodique, Laboratoires Leo S.A., St. Quentin-em-Yvelines, France) 100 UI/100g of body weight. After removing the needle, the small orifice was occluded with a bulldog-type forceps.

Once the cardioplegic and cold saline solution lines were prepared, proximal and distal aortic clamping was performed, followed by transversal aortotomy and the insertion of an 8cm-long 4F catheter (Seldicath®, Laboratoire Pharmaceutique-France)

Incision of the right lumbar vein for a partial examination of the rat and to allow the heart to work with a smaller load, since the animal stops ventilating as soon as the chest is opened and the heart functions in ischemia for 2 to 3 minutes before the cardioplegia is applied. In this situation, therefore, the heart diminishes its contractile force and the same volume that would be well-managed under normal circumstances causes dilation of the ventricular cavities.

Opening and resection of the anterior chest wall by means of bilateral parasternal sectioning, using strong scissors followed by manual luxation of the costovertebral articulations and bilateral diaphragmatic incision. Thus the chest cavity is widely exposed and one proceeds to:

- a) incision of the inferior vena cava;
- b) thymectomy;
- c) left pulmonary arteriotomy to drain the 30 ml of the cardioplegic solution (Solution de Fabiani, Laboratoire Chaix et Du Marais-Faubin, Paris, France).
- d) The left atrium is clamped, and rotation is performed leftwards in order to enable better viewing of the pulmonary artery;
- e) The cardioplegic solution line is opened

- f) Complete release of the lung (this is an important step in the success of the procedure). Once this is done, a beveled cut should be performed with the longer posterior wall;
- g) Release of the ascending aorta, leaving it in a caudal position over the heart repaired with the bulldog forceps;
- h) Dissection and ligation with polypropylene 6-0 (Prolene®, Ethicon®, Johnson & Johnson, Brussels, Belgium) of the superior vena cava proximal to the mouth of the azygos vein, leaving a marker thread;
- i) Dissection and ligation of the inferior vena cava with polypropylene 6-0 (Prolene®, Ethicon®, Johnson & Johnson, Brussels, Belgium);
- j) Release of the atrium attached to the tracheal bifurcation;
- k) The superior vena cava is cut and placed in a caudal position under the heart;
- l) The four pulmonary veins are anchored sutured en bloc;
- m) The heart is finally completely released and placed in 0.9% saline solution 0.9% (sodium chloride (Chlorure de sodium) 0.9%, Fresenius Kabi, Sèvres, France) at 4 °C, with 500 IU of sodium heparine (Heparine Sodique, Laboratoires Leo S.A., St. Quentin-en-Yvelines, France) .

Heterotopic transplantation: modifications in the Ono-Lindsay technique

After repositioning the rat under the surgical microscope:

A) Position of the donor heart:

- I. The heart should be on the surgeon's side the whole time, i.e., in the right lumbar fossa of the recipient rat (Figure 1). This gains time for medullary ischemia, since it avoids manipulating and transporting the heart from the left lumbar fossa to the right, as described in the original Ono-

Lindsay technique (4), which we consider unnecessary, because it increases the time of surgery when the procedure is performed by a single surgeon, and distorts the anatomical structures in mobilizing and transferring the heart from one lumbar fossa to the other.

- II. Horizontal clockwise rotation of 90° of the main axis of the heart, so that the posterior atrial mass (which consists of the en bloc ligation of the 4 pulmonary veins with the left superior vena cava) is maintained – throughout the time of surgery – cranially (in relation to the recipient rat) and not in a posterior position, as was originally described by Ono-Lindsay, while the anterior face of the heart remains turned clockwise, i.e., caudally (Figure 2). Thus, the pulmonary artery – of the transplanted heart – is left facing the inferior vena cava of the recipient on the same plane). In this manner obstruction to the outflow from the right ventricle is avoided, preventing its later thrombosis and distension, resulting in bradycardia – ischemia and arrest of the transplanted heart. This used to occur when the heart was not rotated and the inferior vena cava of the recipient was left on a posterior plane in relation to the pulmonary artery of the transplanted heart. The posterior angulation of the pulmonary artery results in obstruction of the exit from the right ventricle with later thrombosis and biventricular distention.

Performing the anastomoses:

- I. The anastomosis of the pulmonary artery is started in the inferior vena cava. A) the inferior vena cava is not opened until the suture of the posterior margin has been completed, in order to facilitate surgical manipulation; b) anastomoses of the pulmonary artery in the inferior vena cava.

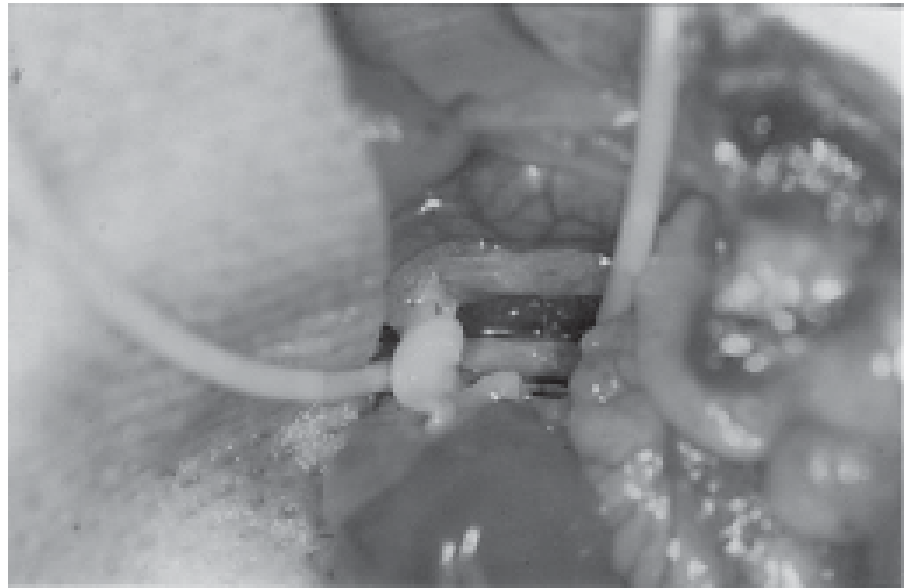


Figure 1 – The arrows mark the aorta and pulmonary artery of the transplanted heart and the aorta of the recipient rat. E= Left; D: right; Cr: cranial; Ca: caudal.

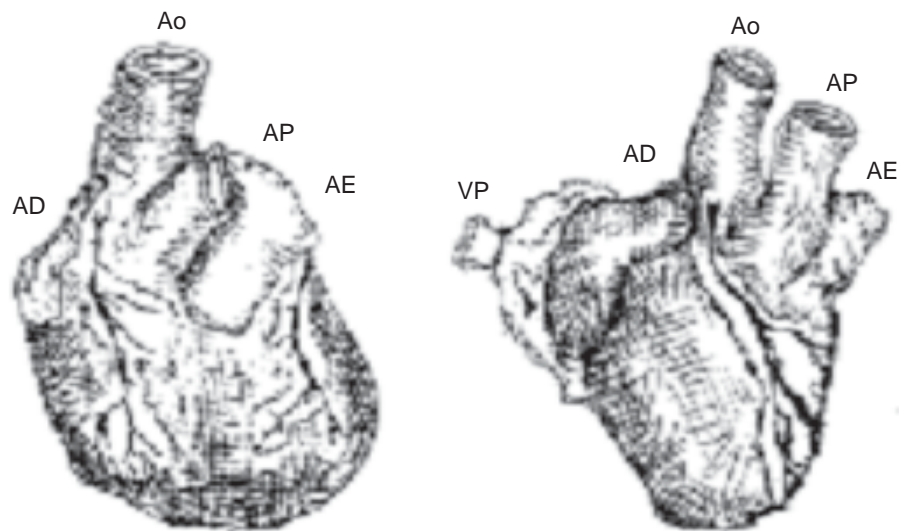


Figure 2 – In this drawing, the horizontal clockwise rotation of 90° of the longer axis of the heart is shown. In the drawing on the left, the heart is in an anteroposterior position and in the drawing on the right, in clockwise rotation. See the four pulmonary veins sutured en bloc, as well as the change of the spatial relationship of the pulmonary vein as compared to the aorta. Ao: aorta; AP: pulmonary artery; AE: left atrium; AD: right atrium; VP: pulmonary veins,.

- II. Anastomosis of the aorta of the donor heart in the abdominal aorta of the recipient is performed using non-sterile 9-0 monofilamentary polyamide, 13 cm long (Polyamide monofilamentar, Ethilon®, Ethicon®, Johnson & Johnson, Bruxelles, Belgium), see Figure 3.

Myocardial protection:

- a) While performing the anastomoses, the myocardium should be protected with a permanent drip of a saline solution cooled to 4 °C;

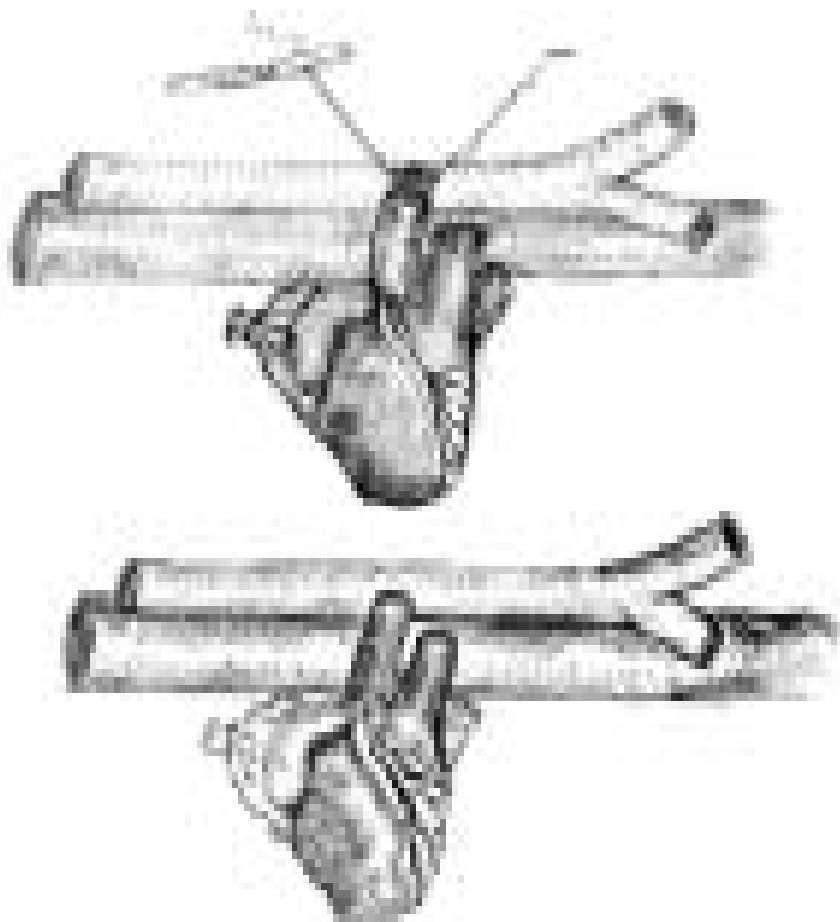


Figure 3 – As shown in the drawing on the left, the margins of the recipient and transplanted aortas are only approached when the suture reaches the caudal commissure. In the figure on the right the two finished sutures.

Postoperative care

The care was the usual experimental surgery laboratory routine under technical staff trained for this purpose, according to French Law (SHARP, 2000; SHARP, 1998).

Slaughtering the animal and anatomopathological study

The ventricular masses of the transplanted and native hearts (the latter with the atrial masses removed) were weighed on a BP 2105 precision scale, Sartorius AG, GÖTTINGEN, Germany.

The shorter and longer axis of the transplanted and native hearts of the surviving rats were measured using a pachymeter. Then the hearts were

placed in formaldehyde at 10% for an anatomopathological study.

Statistical Analysis

The data obtained were arranged in order and tabulated in an EPI-INFO data base.

All the continuous variables are described as mean \pm standard deviation.

The description of the quantitative variables data was arranged in order and tabulated using a polygon of frequencies.

The numerical summary of the results was performed by measures of central trend and dispersion.

The data were organized in Tables and Graphs.

The comparison of the anatomopathological findings between na-

tive and transplanted hearts was performed using the Wilcoxon test for paired samples.

The level of significance (critical $-\alpha$ was established as 5% ($P < 0.05$).

RESULTS

Five rats survived after the first month post-transplantation. They had no problems, and their transplanted heart beat continuously until the time when they were sacrificed.

One hundred and forty-three transplantations were performed during a 10-month period, of which 5 rats lived more than 1 month, and they were sacrificed on the average at 125.6 ± 35.8 days postoperatively. Between the day of the transplantation and the time they were slaughtered, the rats had an average daily weight increase of $1,74 \pm 0,98$ grams.

Sixty-seven rats survived up to 12 hours postoperatively, a mean of 10 ± 6 hours (they all woke up, 23 drank water, but did not feed, 12 walked in the box). Of these 67 rats, 31 died before 6 hours, a mean of 3.4 ± 2.7 hours postoperatively.

Sixty-eight rats died during the first 30 minutes postoperatively, a mean of 24 ± 14.7 minutes. Of these, 21 were the first transplantations and the heart did not beat after the forceps were opened, due to poor myocardial protection. In the 47 remaining ones, an obstructive pulmonary anastomosis was identified in 18 rats and fibrinoid embolism in coronary ostia in 29. The common factor also associated in the 68 rats was excessive bleeding through the suture lines.

Of the 8 that got beyond the first 12 hours postoperatively, 5 survived. The causes of the 3 deaths were unrelated to surgery. The first was due to respiratory arrest because of excessive ether in the anesthesia, in a second surgical procedure (27 hours after transplantation), in order to remove the hemostatic gauze left in while closing after transplantation. The second was a consequence of excessive analgesia during the postoperative period (one of

whose components was acetylsalicylic acid, and having been left well during the night after surgery (4 hours after surgery), the rat was found dead the next morning. There was a lot of blood in the abdominal cavity during the exploratory surgery routinely performed in all rats that awoke from the surgery. The third rat was found dead 28 hours after surgery. No reason was identified. The previous day it had been feeding and moving well around the box and the heart was beating strong and fast (Table 1).

Thus, we had 5 rats that survived after the first month of transplantation. They had no problems, and the transplanted heart beat on until they were sacrificed.

After opening the abdomen of the surviving rats, the hearts beat energetically with a mean frequency of 257±14 beats a minute. The macroscopic aspect of the transplanted hearts was of a major inflammatory reaction with a slightly diminished color of the ventricular mass in both ventricles.

Anatomopathological study of the transplanted hearts

When the rats were slaughtered their weight were an average of 456.4±46.3 grams. The daily weight increase was an average of 0.34± 0.20 grams.

The weight of the ventricular masses of the transplanted hearts was

an average of 1,013±0.27 grams, and of the native hearts 1,260±0.16 grams, P=0.043 (Table 2).

The longer axis of the ventricular masses of transplanted hearts was, on the average 1,12±0.22 millimeters. In the native hearts it was 1.46 ± 0.26 millimeters, P=0.042 (Table 2).

In the transplanted hearts the shorter axis was an average of 1.36 ± 0.48 millimeters and in the native ones it was 1.56±1.27 millimeters, p=0.14 (Table 2).

When comparing the macroscopic aspect of the myocardial masses, it was found that from the 3rd month on there is an intensive rejection phase, shown both in the weight and in the shorter axis of the transplanted heart. This may be observed comparing the macroscopic aspect of the transplanted heart. The weight of the myocardial mass is duplicated from the third month PO, when comparing transplantation nr. 5 with the rest of the myocardial masses of transplantations 1 to 4 (Table 2).

DISCUSSION

In our experiment, the weight of the donor and recipient rat was the same in order to validate the results as well as to make them easier to interpret. There are reports in which donor rats weighing less than the recipients were used (5). For our purposes, this would cause many problems at the time

of analyzing results. But when creating a matrix of the surgical technique, it may be an alternative and could be tested to render technical implementation easier.

The surgical technique initially described by ONO-LINDSAY in 1969 has been used for several studies and experimental models (4). We adapted this technique for use by a single surgeon. For this purpose it was necessary to undertake changes which are described below: a) to maintain the heart in the right lumbar fossa throughout the procedure. In our experience this improved the surgical times and also made it easier to keep the anatomical structures in their correct position and spatial relationship; b) to perform an hourly horizontal rotation of 90° of the longer axis of the heart enabling an improved flow at the exit from the right ventricle, thus avoiding excessive angulation of the trunk of the pulmonary artery in relation to the site of the anastomosis of the inferior vena cava. In our experience, this technical modification improved the results. This occurred to such an extent that the 8 rats that lived over 24 hours postoperatively presented this technical variation; c) to make the posterior margin of the anastomosis, between the pulmonary artery of the donor heart with the recipient's inferior vena cava. The latter, without the longitudinal cavotomy, improved our time of medullary ischemia by 9 minutes, and also elim-

Table 1 – Analysis of survival

	Group I	Group II	Group III	Group IV	Group V
Hours postoperatively	No heartbeat ¹	≤ 30' ²	<6 hs ³	> 6 hs < 12hs ⁴	> 12 hs
Cause of death	Poor myocardial protection	Obstructive pulmonary anastomosis/ fibrinoid embolism in coronary ostia	Thrombi in the RV/Excessive bleeding	Paraplegia/ Excessive bleeding	Problems of analgesia and anesthesia
Number of rats	21	47	31	36	8

1. Poor myocardial protection, use of hot light with rapid heating of the myocardium.
2. Arrest of the donor heart due to fibrinoid embolism in the coronary ostia (rapid ischemic change of myocardial color) or obstructive anastomosis of the pulmonary artery (caused by the early and excessive distension of the right atrium and coronary veins).
3. A thrombus was found in the right ventricle associated with excessive bleeding (more than 4 swabs), in 12 rats. In the 12 remaining ones of the group there was excessive bleeding (more than 4 swabs).
4. Of those who died after 6 hours and before 12 hours, the main cause was excessive bleeding (more than 4 swabs in each case). In 15 it was associated with paraplegia.
5. Of the 8 that survived beyond 12 hours, 5 are alive and all have survived for over one month.

Table 2 – Anatomopathological study of transplanted hearts.

Rats n=5	Time of TX (days)	Ventricular Native (gr)	Mass TX (gr)	Longer Native (cm)	Axis TX (cm)	Shorter Native (cm)	Axis TX (cm)	Weight of rat during surgery (g)	Weight of slaughtered rat (g)
R1	188	1.324	1.285	1.9	1.5	3.8	4.0	224	515
R2	118	1.201	1.023	1.4	0.9	0.9	0.6	250	495
R3	113	1.489	1.062	1.2	1.1	0.9	0.9	230	410
R4	113	1.256	1.135	1.4	1.1	1.1	0.8	240	442
R5	96	1.031	0.561	1.4	1.0	0.9	0.5	240	420
Mean	125.6	1.260	1.013	1.46	1.12	1.56	1.36	236.8	456.4
SD	35.8	±0.16	±0.27	±0.26	±0.22	±1.27	±0.48	±10.05	±46.3
P=*		0,043		0,042		0,140		0,042	

* Wilcoxon matched pairs signed ranks test.

inated bleeding at this level of the suture. All surviving rats presented this technical variation.

As shown in Table 2, during the preparatory phase of the LV of the transplanted hearts, there is a hypotrophy of the left ventricular mass. This is characterized by reduction in the weight of ventricular masses, the longer axes and the shorter axes of the transplanted hearts as compared to the native hearts (Table 2), although it is not significant in the measure of the shorter axis or reduction.

The mechanical work discharge provokes a rapid, predictable degree of hypogrowth of the myocardial mass followed by atrophy (5), as well as a rapid, severe deterioration of the left ventricular diastolic and systolic function during the first 24 to 48 hours of pressure discharge (6).

Functionally, it is characterized by diminished velocity and contraction force (6,7). Structurally, the discharge is characterized by diminished myocardial mass and synthesis of the structural proteins, in the abdominal heterotopic cardiac model (8). The genetic consequences of the discharge of mechanical work in the myocardium have not been well studied (9). There is a re-expression of the fetal genes program, constituting a specific and dynamic adaptive mechanism of the myocardial atrophy. The consequences are exchanges between the adult protein isoforms and the fetal protein isoforms, as well as the re-expression of the protooncogenes c-fos, c-myc, c-jun, c-jun b (10). The capacity and efficacy of the

protein synthesis decreases as a consequence of diminishing the 18S ribosomal and ARN ribosomal content, of 53% and 48% at 7 and 14 days, respectively, relating to the recipient heart (8). We must emphasize the concept that lack of left ventricular preparation is a dynamic adaptive mechanism, as shown in literature (10,8,6) (Figure 4).

Attempts at using an intraventricular balloon to perform hemodynamic measures during retraining were frustrated by complications that rendered this technique unfeasible.

These complications, added to the observations of the position of the posterosuperior aspect of the left atrium on a posterior plane and right below the posterior aspect of the inferior vena cava, inspired our idea of creating a more physiological experimental model for left ventricular retraining, which is observed in Figures 5 and 6. Technically, it consists of adding the anastomosis of the inner aspect of the left atrium of the posterior aspect of the inferior vena cava, during a second surgical procedure 15 days after transplantation, and the inferior vena cava of the recipient rat will be ligated proximally to the transplanted heart, permitting the retraining of the left ventricle, i.e., all of the flow of the inferior vena cava performs the left atrium-left ventricle-aorta circuit, submitting again to the left ventricle considering the systemic loads. The initial results were encouraging. Thus, the deleterious histological effects such as severe and extensive necrosis due to

the intraventricular balloon's contact on the endocardial surface observed by Galíñanes (6) can be avoided.

Concluding, the experimental model for abdominal heterotopic cardiac transplantation in rats, to evaluate hypotrophy and retraining of the LV is technically feasible, although it requires an extensive training phase.

The transplanted animals have a low rate of survival, and the causes of mortality detected were: deficient myocardial protection, coronary embolism, obstruction of pulmonary anastomosis, thrombosis of the right ventricle, paraplegia and bleeding.

In transplanted hearts, the unpreparedness or hypotrophy was shown by the reduction of the ventricular mass, as well as by the reduction of the longer and shorter axes of the left ventricle.

ACKNOWLEDGEMENT

The authors acknowledge the sponsorship of Prof. Claude Planché and scientific orientation by Prof. Alain Serraf, from the Centre de Chirurgie Marie-Cannclougue, Le Plessis-Roginon, France during the conduction of this project. Research grants were received from Conselho Nacional de Desenvolvimento Científico e Tecnológico – CNR and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul – FAPERGS.

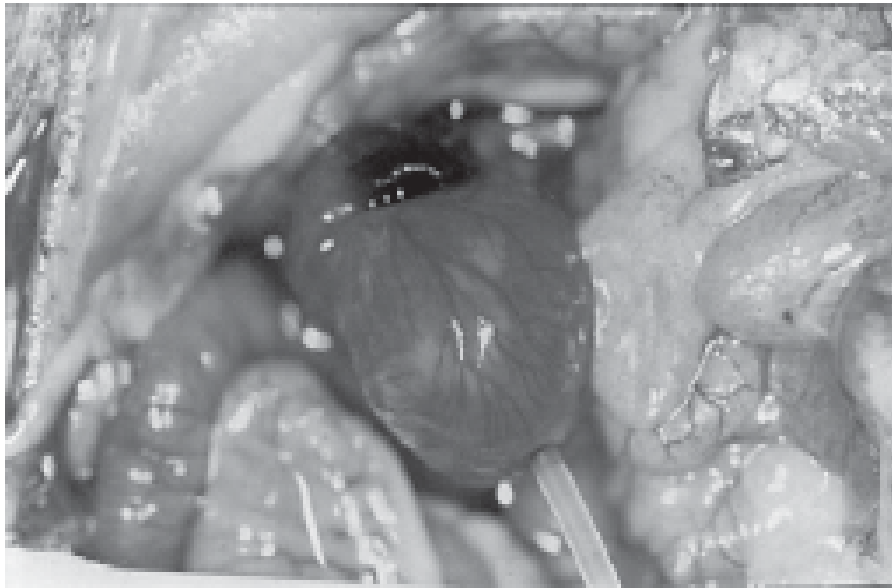


Figura 4 – Heart with good function after opening the ligatures. Good myocardial color, good spatial position of the great heart vessels in relation to the ventricular mass.

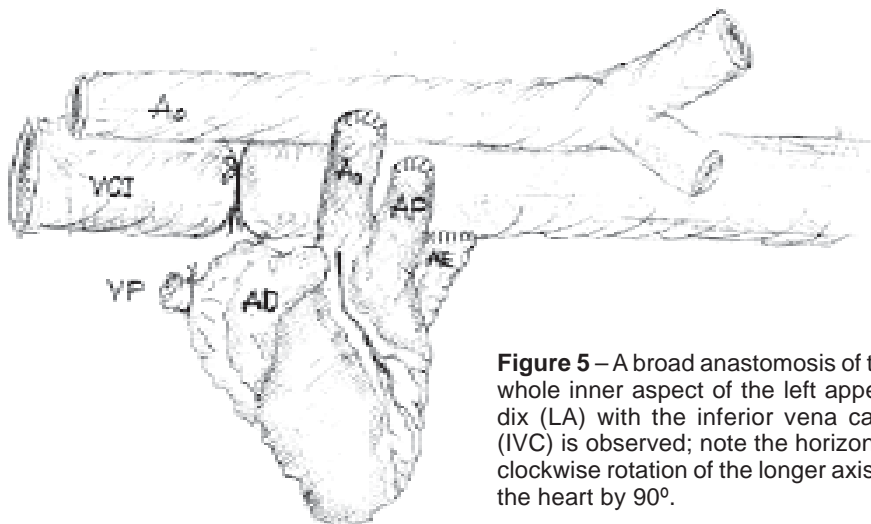


Figure 5 – A broad anastomosis of the whole inner aspect of the left appendix (LA) with the inferior vena cava (IVC) is observed; note the horizontal clockwise rotation of the longer axis of the heart by 90°.

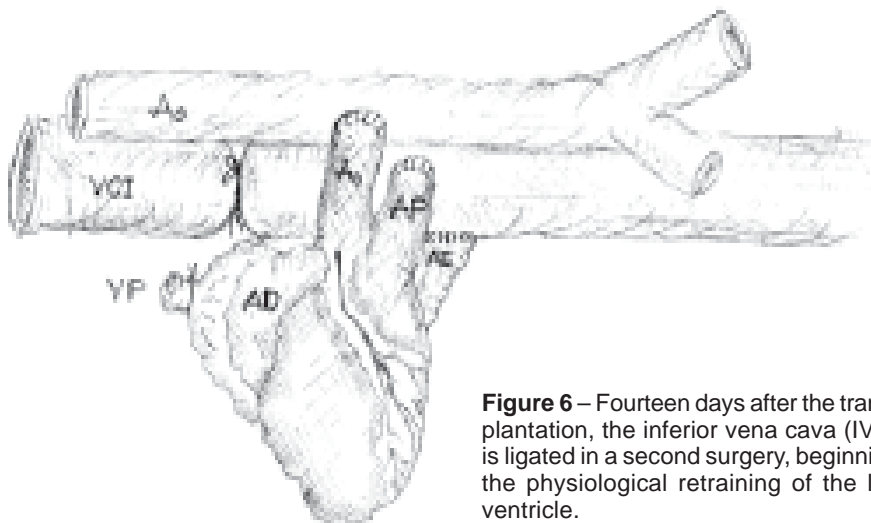


Figure 6 – Fourteen days after the transplantation, the inferior vena cava (IVC) is ligated in a second surgery, beginning the physiological retraining of the left ventricle.

REFERENCES

1. CASTAÑEDA AR, JONAS RA, MAYER JE, HANLEY FL. Cardiac surgery of the neonate and infant. Philadelphia: W.B. Saunder Co., 1994.
2. YACOB M, RADLEY-SMITH R, MC CLaurin R. Two-stage operation for anatomical correction of transposition of the great arteries with intact ventricular septum. *Lancet* 1977; 1:1275-78.
3. YACOB M, BERNHARD A, LANGE P et al. Clinical and hemodynamic results of two-stage anatomic correction of simple transposition of the great arteries. *Circulation* 1980; 62(Suppl.I):II90-6
4. ONO K, LINDSEY E. Improved technique of heart transplantation in rats. *Journal of Thoracic Cardiovascular Surgery* 1969; 57(2):225-229.
5. KLEIN I, SAMAREL AM, WELIKSON R, HONG CH. Heterotopic cardiac transplantation decreases the capacity for rat myocardial protein synthesis. *Circulation Research* 1991; 68:1100-1107.
6. GALIÑANES M, HEARSE DJ. Metabolic, functional, and histologic characterization of the heterotopically transplanted rat heart when used as a model for the study of long-term recovery from global ischemia. *Journal Heart Lung Transplantation* 1991; 10:79-91.
7. GALIÑANES M, ZHAI X, HEARSE DJ. The effect of load on atrophy, myosin isoform shifts and contractile function: studies in a novel rat heart transplant preparation. *Journal of Molecular and Cellular Cardiology* 1995; 27:407-417.
8. KLEIN I, HONG C, SCHREIBER SS. Isovolumic loading prevents atrophy of the heterotopically transplanted rat heart. *Circulation Research* 1991; 69:1421-1425.
9. GEENEN DL, MALHOTRA A, BUTTRICK PM. Ventricular pacing attenuates but does not reverse cardiac atrophy and an isomyosin shift in the rat heart. *Annals Journal of Physiology* 1994; 267:H2149-H2154.
10. DEPRE C, SHIPLEY GL, CHEN W et al. Unloaded heart in vivo replicates fetal gene expression of cardiac hypertrophy. *Nature Medicine* 1998; 4(11): 1269-1275.