Modified Multivisceral Transplantation with Native Spleen Removal in Rats

Pablo Stringa[1,2] Nidia Monserrat Arreola[3] Ane M. Andres Moreno[4] Carlota Largo[5] Martín Rumbo[6] Francisco Hernandez[1,4]

[1] Cirugía Experimental, Fundacion Investigacion Biomedica Del Hospital Universitario La Paz, Madrid, Spain
[2] Instituto de Medicina Traslacional, Trasplante y Bioingeniería (IMETTYB), Universidad Favaloro-CONICET, Buenos Aires, Argentina
[3] Hospital de Pediatría, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Mexico
[4] Cirugía Pediátrica, Hospital Universitario La Paz, Madrid, Spain
[5] Cirugía Experimental, Hospital Universitario La Paz, Madrid, Spain
[6] Instituto de Estudios Inmunológicos y Fisiopatológicos (IIFP-CONICET-UNLP), Universidad Nacional de La Plata, La Plata, Buenos Aires, Argentina

Address for correspondence Francisco Hernandez, MD, PhD, Department of Pediatric Surgery, Hospital Universitario La Paz, Paseo de La Castellana, 261 Madrid 28046, Spain (e-mail: fhernandezo@salud.madrid.org).

Eur J Pediatr Surg

Abstract

Background Modified multivisceral transplantation (MMVTx) refers to the use of a graft that includes all abdominal organs except the liver. The use of this type of transplant in children and adults expanded over the last years with good results. However, long-term survival in experimental models has not been reported. Our aim is to describe in detail some technical modifications of MMVTx to obtain long-term survival.

Materials and Methods Syngeneic (Lewis–Lewis) heterotopic MMVTx was performed in 16 male rats (180–250 g). All procedures were performed under isoflurane anesthesia. The graft consisted of stomach, duodenopancreatic axis, spleen, and small bowel. The vascular pedicle consisted of a conduit of aorta, including the celiac trunk and the superior mesenteric artery (SMA), and the portal vein (PV). The engraftment was performed by end-to-side anastomosis to the infra-renal cava vein and aorta. After reperfusion, the graft was accommodated in the right side of the abdomen, and a terminal ileostomy performed. The native spleen was removed.

Results Donor and recipient time was 39 ± 4.4 minutes and 69 ± 7 minutes, respectively; venous and arterial anastomosis time was 14 ± 1 minutes and 12.3 ± 1 minutes, respectively. Total ischemia time was 77.2 ± 7.9 minutes. Survival was 75% (12/16), six were sacrificed after 2 hours, and six were kept alive for long-term evaluation (more than 1 week).

Conclusion Long-term survival is reported after heterotopic MMVTx in rats. The heterotopic MMVTx with native spleen removal would potentially improve the existent models for transplant research. The usefulness of this model warrants further confirmation in allogeneic experiments.

Keywords
- modified multivisceral transplantation
- multivisceral transplantation
- rats
- spleen
- intestinal transplantation

received
October 5, 2017
accepted after revision
January 16, 2018

DOI https://doi.org/10.1055/s-0038-1632375.
ISSN 0939-7248.

Introduction

Modified multivisceral graft refers to a composite graft that includes stomach, duodenopancreatic axis, and the small bowel; the colon can be included or not; and by definition, the liver is not included by contrast with the so-called multivisceral graft.

The use of modified multivisceral transplantation (MMVTx) graft expanded in the last years both in adults and children with good results. Patients with chronic intestinal failure with preserved liver function are candidates for this type of transplant, mostly patients affected with polyposis and motility disorders.[1,2] The international intestinal transplant registry identified that the proportion of grafts without the liver component has significantly increased over the last years; better care of patients suffering from intestinal failure and particularly the use of new lipid emulsions are responsible of this trend.[3–5] On the other hand, the inclusion of the liver in the graft has shown immunological advantages and improved survival, but given the scarcity of donors, the use of the liver just for immunological reasons would not be justified.[6]

In opposition to the clinical success, MMVTx is a poorly explored area in experimental models, only one paper from Galvao et al[7] described the technique in rodents without long-term survival. MMVTx graft offers several advantages, as all abdominal viscera except the liver are included, with the corresponding immunologic load. One the other hand, the potential immunological benefits of the liver could act as a confounding variable; therefore, the exclusion of the liver magnifies the interest of MMVTx as an experimental model in the field of transplantation research.

The spleen plays an important role in the appearance of immunological complications, such as graft versus host disease (GVHD). Different modifications in the experimental model by keeping in place or removing the native and the graft spleen could be helpful to further elucidate the mechanism of this effect.

Our aim was to describe in detail some technical modifications of MMVTx to obtain long-term survival, as a necessary step to use the model for immunological studies. Furthermore, we report main complications associated with the procedure and highlight the value of this experimental model for the study of different immunological processes related with MMVTx.

Materials and Methods

Animals' Use and Care

Adult Lewis rats (180–250 g in weight) with similar body weight were paired as donor and recipients. Animals were housed individually in a climate-controlled room (21 ± 2°C and relative humidity of 45 ± 15%) on a 12-hour light–dark cycle at our institution's animal facilities. Rat chow and water *ad libitum* had been provided for 1 week of adaptation. Before the experiments, they had been fasted for 24 hours with 5% glucose—normal saline and water ad libitum. The local ethics committee approved all experiments. This study was performed in strict accordance with the recommendations in the European Union Criteria for Animal Use in Scientific Experimentation (63/2010 EU) and related Spanish legislation (RD 53/2013). The protocol was approved by the Animal Welfare Ethics Committee of La Paz University Hospital (PROEX 014–2017)

Heterotopic syngeneic MMVTx were performed in 16 pair of rats following the technique describe below.

Procedures

All procedures were performed under general anesthesia induced by isoflurane 3 to 5% on standard atomizer. Animals were weighted, and the abdomen was shaved and cleaned with povidone–iodine. Tail vein was catheterized (24G catheter) for intravenous (iv) one-fifth saline infusion (3–5 cc/h); alternatively, 5 mL of one-fifth saline was administered subcutaneously before the abdominal incision. A single dose of tramadol hydroclorhidrate (30 mg/kg sc) was administered as analgesic. Isoflurane was reduced to 2 to 2.5% after performing the xipho-pubic laparotomy. Warm blankets were used for both donor and recipient during the procedure. Midline incisions were performed, and the abdominal cavity was exposed by small hook retractors. The remaining procedures were performed under surgical microscope vision (×4, ×10, or ×25 as appropriate). Bipolar coagulation was used for all vessels except for the aorta, reducing the operative time that requires the use of ties.

Donor

Physiologic malrotation was corrected, and the small bowel was exteriorized to the left side of the laparotomy. The duodenojejunal ligament was divided toward the superior mesenteric artery (SMA). Then, total colectomy was completed after coagulation of ileocolonic and colonic vessels, and the distal small intestine was transected 3 cm proximal to the ileocecal valve. Then, the gastric vessels were coagulated at the level of the esophagogastric junction, and the esophagus was transected. The spleen and pancreas were mobilized to the right to reach the aorta; special care was required at this part to avoid inadvertent damage of the SMA. The proximal portions of the superior mesenteric vein and portal vein (PV) were dissected from the ligament up to the porta hepatis, and the hepatic artery and bile duct were coagulated. The small intestine was placed in the left side of the abdomen, and the SMA and celiac trunk were dissected up to the abdominal aorta (►Fig. 1). The abdominal aorta was clamped above the celiac trunk, and the graft was perfused in situ with heparinized (20 IU/mL) lactate Ringer's solution at 4°C (injected gently by direct syringe infusion, 5 mL) through a catheter inserted into the abdominal aorta until the drainage fluid from the cut end of the PV was clear. The aorta was transected below the SMA and above the celiac trunk after the proximal end was tied with Prolene 6/0. The harvested graft with a vascular pedicle, consisting of an aortic segment (containing the SMA and celiac trunk) and the PV, was stored at 4°C in lactate Ringer's solution. The aortic clamp was released, and the donor animal was sacrificed by exsanguination.

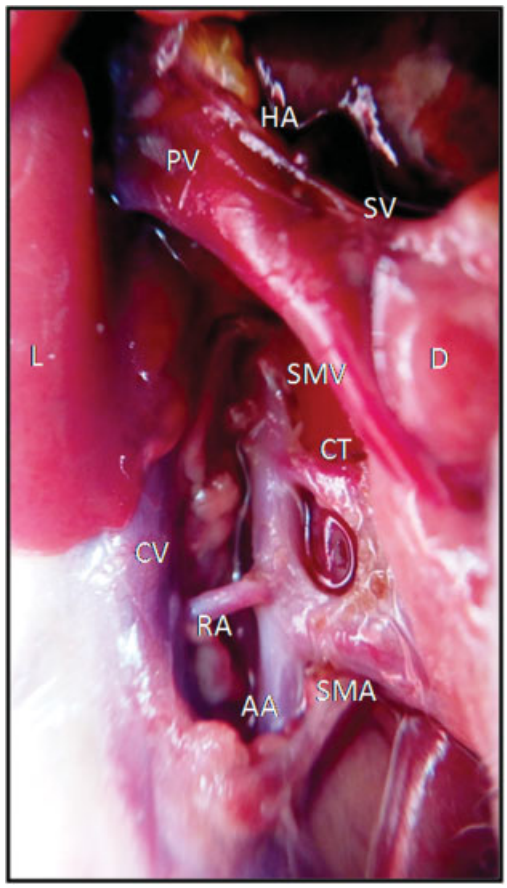

Fig. 1 Donor dissection before graft perfusion. AA, abdominal aorta; CT, celiac trunk; CV, cava vein; D, duodenum; HA, hepatic artery; PV, portal vein; RRA, right renal artery; SMA, superior mesenteric artery; SMV, superior mesenteric vein; SV, splenic vein.

Back Table

The viability of the graft is evaluated during this phase (►Fig. 2A). The presence of reddish areas in the small bowel reflects insufficient perfusion or suffering of the graft during the procurement. The graft was discarded if reddish areas were extended through a significant part of the graft. The pedicles were identified and checked for open branches or damages (►Fig. 2B). The PV was referred or not (9/0 monofilament suture) according to the surgeon preferences. The proximal half of the stomach was sectioned after 6/0 monofilament tie or suture (►Fig. 2C).

Recipient

The abdominal aorta and infrarenal vena cava were exposed and dissected to allow safe clamping during the procedure (►Fig. 3A). After aortic longitudinal arteriotomy, the lumen was irrigated with heparinized Ringer's solution. The graft was placed on the right side of the recipient, surrounded by gauze soaked in cool Ringer's solution. The venous anastomosis was performed between the graft PV and the infrarenal vena cava of the recipient using running 9–0 monofilament suture (►Fig. 3B). The PV was clamped and the cava vein released, if this maneuver is technically difficult, both the cava vein and aorta can be released at the end of the arterial anastomosis (►Fig. 3C). The arterial anastomosis between graft aorta and recipient aorta was performed in the same fashion (►Fig. 3C and D). After the patency of the anastomosis was confirmed, the blood supply to the graft was reestablished removing the venous clamp first and then the arterial clamp (►Fig. 4). The graft was accommodated in the right side of the abdomen. Native spleen is removed (►Fig. 5). The distal end of the graft was used for terminal ileostomy using 7/0 monofilament suture (►Fig. 6). Laparotomy was closed with running monofilament 4/0 suture. Additional iv or subcutaneous (sc) fluid was administered if significant bleeding occurred after revascularization. Then inhalatory anesthesia was discontinued while oxygen supply was maintained until the animal awoke. Subcutaneous analgesics were administered, and the animal was moved back to the cage.

As we previously reported for isolated intestinal transplantation in rats, intra-surgical complications, such as severe arterial bleeding, irreversible portal thrombosis or stenosis, and inadequate graft reperfusion, among others, were causes of applying the endpoint criteria and interrupt the surgical procedure.[8]

Postoperative Care

Ceftriaxone (70 mg/kg/24 h sc) had been used for 5 days and tramadol hydroclorhidrate (30 mg/kg/24 h sc) for 72 hours. Animals were checked every 12 hours in search of signs of pain and/or discomfort. As we previously reported, a standardized quantitative score for clinical monitoring of recipient was used.[9] Briefly, animal weight, ocular secretion, hair appearance, and posture, among others parameters, were considered. Each recipient received a general score resulting from adding each evaluated parameter. Endpoint criteria were applied in animals with persistent signs of pain and/or discomfort.

Experimental Design

Immediate and long-term MMVTx survival was assessed. Also, macroscopic and microscopic appearance of the graft to validate the surgical technique was studied at different post-reperfusion times.

Transplanted organs (stomach, pancreas, and small bowel) and native target organs for graft versus host disease (GVHD) and graft rejection (skin, liver, lung, and small bowel) were sampled for histopathological analysis. All samples were fixed in formalin 10% and were stained for hematoxylin-eosin examination.

Results

Donor and recipient time was 39 ± 4.4 minutes and 69 ± 7 minutes, respectively; venous anastomosis time was 14 ± 1 minutes, and arterial anastomosis time was 12.3 ± 1 minutes. Total ischemia time was 77.2 ± 7.9 minutes.

Inadequate perfusion of the graft (considered as a remnant of blood in the graft after washing) was observed in two donors who were not included in the study. Recipient immediate surgical procedure survival was 75% (12/16).

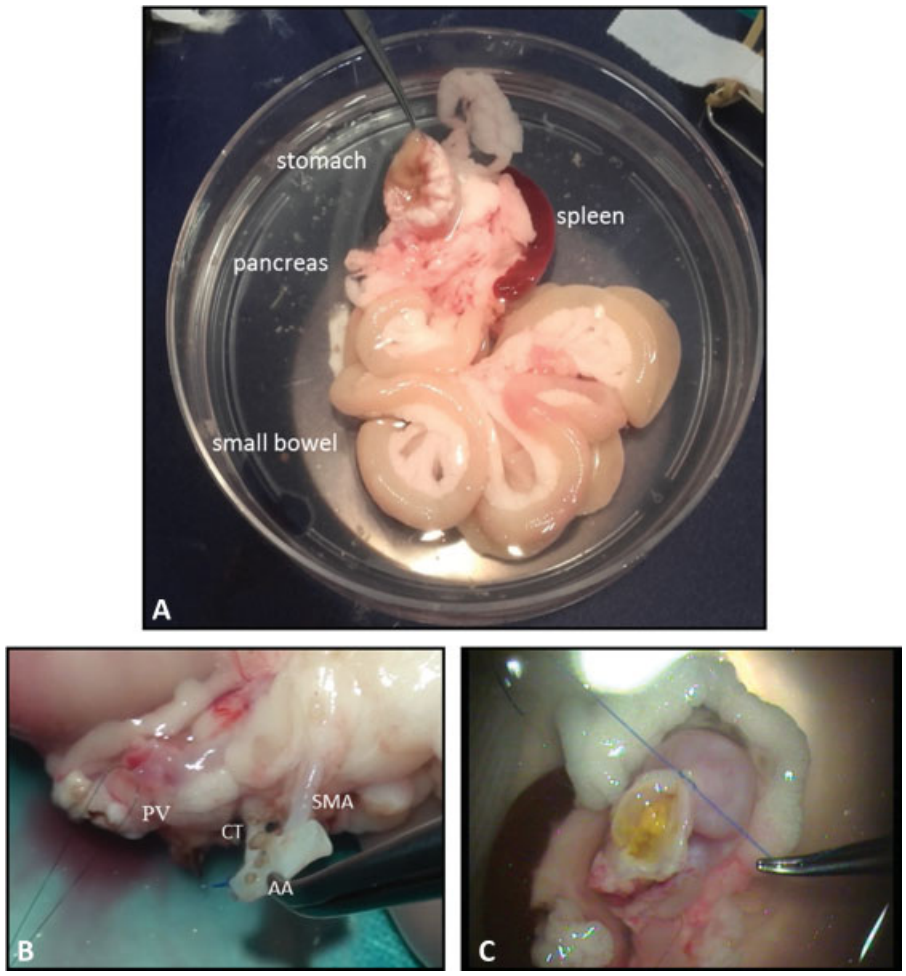

Fig. 2 General and detailed aspects of the graft during the back table procedure. (**A**) Modified multivisceral graft in cold lactate ringer solution. (**B**) Detail of graft pedicles. (**C**) Upper gastric section and suture. AA: abdominal aorta conduit including superior mesenteric artery (SMA) and CT, celiac trunk; PV, portal vein.

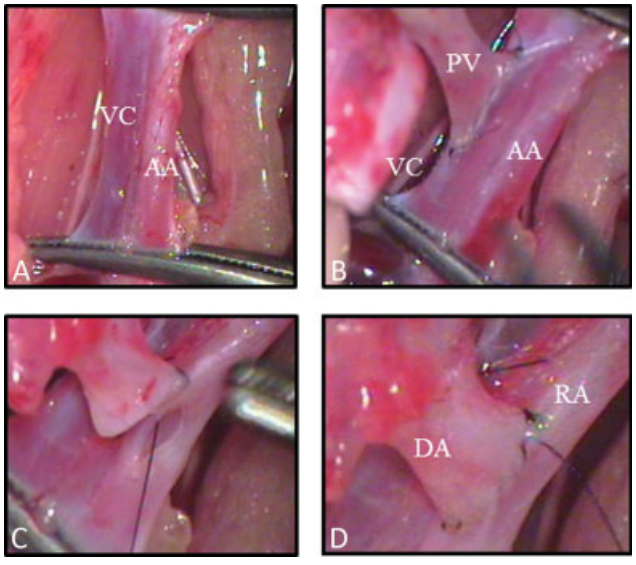

Fig. 3 Preparation of the recipient's abdominal vessels for vascular anastomoses (**A**). Venous anastomosis completed (**B**). End-to-side arterial anastomoses between the cuff of the DAA and the RAA (**C** and **D**). AA, abdominal aorta; DAA, donor aorta; PV, portal vein; RAA, recipient infrarenal aorta; VC, vena cava.

Causes of death were as follows: arterial bleeding (2), portal thrombosis (1), and portal stenosis (1). Minor complications related to the ostomy were observed in three animals: bleeding (2) and spontaneous closure (1). In recipient sampled 2 hours after reperfusion ($N = 6$), a good graft revascularization was observed at both macroscopic and microscopic level (►Fig. 4A–E). Of the remaining six survivors, one died after 24 hours (undetermined cause), and five survived for more than 1 week and were fully sampled (two-fifth were sacrificed at 1 week, two-fifth at 2 weeks, and one-fifth at 3 weeks). Regarding the clinical status, recipients showed mild piloerection and weight loss during the first 48 hours after transplantation. From the third day after MMVTx, the animals recovered their original weight and showed no signs of discomfort or pain.

Histology showed no signs of rejection, GVHD, or ischemic damage in native or graft intestine. Normal histology was also observed in native liver, lungs, and skin.

Discussion

Intestinal transplantation in rats is widely acknowledged as a very challenging procedure. However, the unique

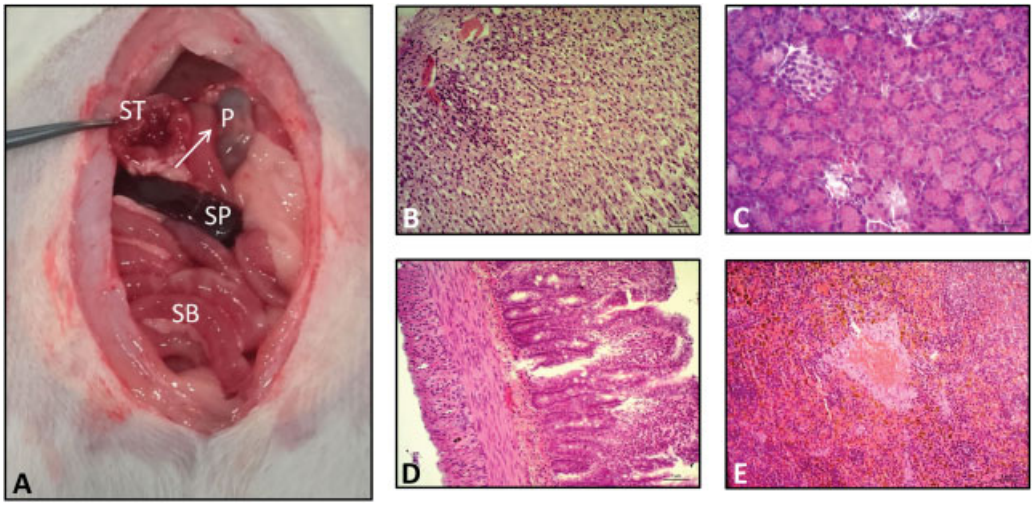

Fig. 4 Macroscopic appearance of MMVTx graft during immediate reperfusion showing adequate revascularization. To observe gastric mucosa reperfusion the stomach was not sutured in this case (**A**). Normal microscopic appearance of transplanted stomach (**B**), pancreas (**C**), small bowel (**D**), and spleen (**E**) are also shown.

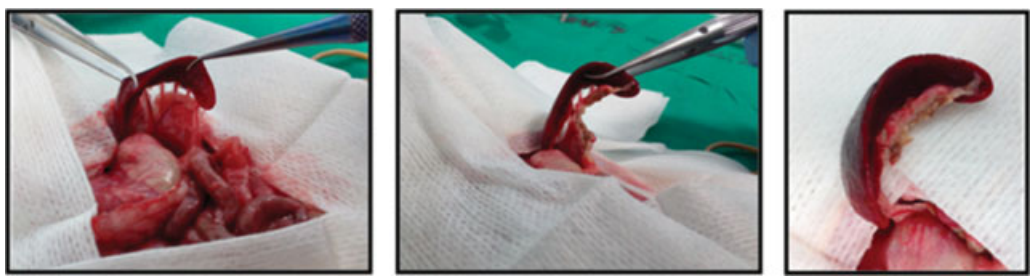

Fig. 5 After graft reperfusion, native spleen is removed.

immunological properties of the small bowel justify the use of this model instead of other solid organ transplantation in transplant immunology research. The high lymphocyte load contained in the intestinal graft accounts for the appearance of GVHD and other immunological complications more often related to bone marrow transplantation; in fact, GVHD is anecdotic after other solid organ transplants. In the clinic, GVHD was found in 10% of cases showing 55% mortality.[10,11] In a classic paper, Monchik and Russel described the occurrence of GVHD-like phenomenon using Lewis to Brown Norway F_1 hybrid strain combination. Murase et al[12] and Galvao et al[13,14] further developed the experimental model of GVHD in the following years; however, these models were based in the genetic immunologic of the different strain combinations. This scene is probably different from what we found in the clinic; therefore, a new model based in the lymphocyte load rather than the genetic predisposition to GVHD would be helpful in the understanding of this severe complication. The MMVTx proposed in this paper aims to become this new model, offering the larger immunological load, without the protective effects of the inclusion of the liver.

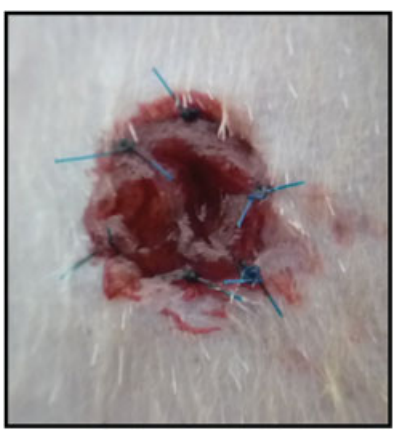

Fig. 6 Macroscopic aspect of terminal ileostomy after modified multivisceral transplantation in rat.

Galvao et al previously published the feasibility of MMVTx in rats. However, no long-term survival was reported being the current paper the first describing this outcome. Apart from survival, there are major technical differences between both procedures as the group of Galvao described what could be called orthotopic MMVTx, meaning that the graft occupies the place of the native organs, and we described an heterotopic

model, as the native organs (except for the spleen) were kept in place.[7] The use of orthotopic or heterotopic model in the experimental setting is a recurrent controversy. Our group opted generally for the orthotopic model in our previous intestinal transplant research, as it reproduces the clinical situation and the physiology of the intestine.[15–17] On the other hand, the advantages of the heterotopic model should not be denied; first, survival is higher as the intestinal continuity is not restored, and the changes of infection are lower; second, native small bowel enterectomy is avoided, which also favors long-term survival. The discussion would not be closed, but we could summarize that the orthotopic model should be used to study the real physiology of intestinal transplantation, and the heterotopic model would be recommended when infection need to be ruled out, and only immunological phenomenon are to be considered.

Besides the heterotopic position of the graft, others surgical aspect had to be considered during the development of this novel experimental transplant. Due to the limited evidence in experimental models of MMVTx in rats,[7] we have used our previous experience in rodents experimental surgery and the extensive literature published in isolated small bowel and multivisceral transplantation to transfer to our heterotopic MMVTx model as well as pre- and post-surgical animal handling, anesthetics, analgesics, and antibiotics, with a very good effectiveness.[8,9,15–20] Focusing on vascular anastomosis, we performed an end-to-side arterial anastomosis between the donor and recipient aorta following the technique reported by Galvao et al. In contrast, due to the heterotopic graft position, we performed a hand-to-sewn venous anastomosis between donor PV and recipient cava vein instead of the described cuff technique.[7]

In agreement with the multicenter work published by our surgeons group in isolated intestinal transplantation in rats,[8] main complications observed during recipient MMVTx surgery were related to the arterial and venous anastomosis confection. These results allow to conclude that a correct microsurgical technique to perform vascular anastomosis represents a key point to success.

After reperfusion, the entire graft is positioned in the right abdomen. This location was adopted from our previous experience and from other researchers in heterotopic intestinal transplantation. As shown in ►Fig. 4, this position allows a good graft reperfusion, and, on the other hand, it is not necessary to manipulate native organs, such as stomach, pancreas, and duodenum. In the same way, terminal ileostomy was performed following reported techniques.[9,18,19]

Other important contribution in the MMVTx model is the inclusion of the spleen in the graft and the native spleen removal. In the clinic, the spleen is no longer included in the graft because the risk of GVHD is increased. Regarding the native spleen, preservation is paramount to avoid the complications of the asplenic state and to reduce the risk of GVHD. The technical modification to preserve native spleen together with duodenum and pancreas was described first in MMVTx[21] and some years later, without the duodenopancreatic axis and with some additional maneuvers in MVTx.[22] Based on the clinical experience, in our experimental model, we decided to include the spleen in the graft and to remove the native spleen as means to increase the rate of GVHD.

To summarize, a long-term survival is reported after MMVTx in rats. Besides, the heterotopic MMVTx with native spleen removal would potentially improve the existent models to reproduce GVHD and other immunologic complications related to solid organ transplantation. The usefulness of this model warrants further confirmation in allogeneic experiments

Conflict of Interest

None.

References

1 Cruz RJ Jr, Costa G, Bond GJ, et al. Modified multivisceral transplantation with spleen-preserving pancreaticoduodenectomy for patients with familial adenomatous polyposis “Gardner’s Syndrome”. Transplantation 2011;91(12):1417–1423
2 Takahashi H, Delacruz V, Sarwar S, et al. Contemporaneous chronic rejection of multiple allografts with principal pancreatic involvement in modified multivisceral transplantation. Pediatr Transplant 2007;11(04):448–452
3 Torres C, Sudan D, Vanderhoof J, et al. Role of an intestinal rehabilitation program in the treatment of advanced intestinal failure. J Pediatr Gastroenterol Nutr 2007;45(02):204–212
4 Goulet O, Joly F, Corriol O, Colomb-Jung V. Some new insights in intestinal failure-associated liver disease. Curr Opin Organ Transplant 2009;14(03):256–261
5 Goulet OJ. Intestinal failure-associated liver disease and the use of fish oil-based lipid emulsions. World Rev Nutr Diet 2015;112:90–114
6 Grant D, Abu-Elmagd K, Mazariegos G, et al; Intestinal Transplant Association. Intestinal transplant registry report: global activity and trends. Am J Transplant 2015;15(01):210–219
7 Galvão FH, Waisberg DR, Cruz RJ Jr, Chaib E, Carneiro D’Albuquerque LA. Modified multivisceral transplantation in the rat. Transplantation 2013;96(02):e3–e4
8 Stringa P, Andres AM, Lausada N, et al. Small bowel transplantation in rats, a multicener experience summarizing the pitfalls to be overcome. Trends Transplant 2017;10(01):1–7
9 Stringa P, Romanin D, Lausada N, et al. Gut permeability and glucose absorption are affected at early stages of graft rejection in a small bowel transplant rat model. Transplant Direct 2017;3(11):e220
10 Andres AM, Santamaría ML, Ramos E, et al. Graft-vs-host disease after small bowel transplantation in children. J Pediatr Surg 2010;45(02):330–336
11 Wu G, Selvaggi G, Nishida S, et al. Graft-versus-host disease after intestinal and multivisceral transplantation. Transplantation 2011;91(02):219–224
12 Murase N, Demetris AJ, Woo J, et al. Graft-versus-host disease after brown Norway-to-Lewis and Lewis-to-Brown Norway rat intestinal transplantation under FK506. Transplantation 1993;55(01):1–7
13 Galvao FH, Murase N, Todo S, et al. Cytokine profile in graft-versus-host disease after small bowel transplantation. Transplant Proc 1996;28(05):2455
14 Galvao FH, Ye Q, Doughton C, et al. Experimental animal model of graft-versus-host disease (GVHD) after small-bowel transplantation: characteristics of the model and application to developing treatment strategies. Transplant Proc 1997;29(1-2):700
15 Hernández F, Zou Y, López G, et al. Is portal venous outflow better than systemic venous outflow in small bowel transplantation? Experimental study in syngeneic rats. J Pediatr Surg 2005;40(02):336–340
16 Wang Z, Hernandez F, Pederiva F, et al. Ischemic preconditioning of the graft for intestinal transplantation in rats. Pediatr Transplant 2011;15(01):65–69

17 Zou Y, Hernandez F, Burgos E, et al. Bacterial translocation in acute rejection after small bowel transplantation in rats. Pediatr Surg Int 2005;21(03):208–211
18 Andres AM, Santamaria M, Hernandez-Oliveros F, et al. Difficulties, guidelines and review of developing an acute rejection model after rat intestinal transplantation. Transpl Immunol 2016; 36:32–41
19 Foell D, Becker F, Hadrian R, Palmes D, Kebschull L. A practical guide for small bowel transplantation in rats-review of techniques and models. J Surg Res 2017;213(213): 115–130
20 Murase N, Demetris AJ, Furuya T, Todo S, Fung JJ, Starzl TE. Comparison of the small intestine after multivisceral transplantation with the small intestines transplanted with portal or caval drainage. Transplant Proc 1992;24(03):1143–1144
21 Abu-Elmagd KM. Preservation of the native spleen, duodenum, and pancreas in patients with multivisceral transplantation: nomenclature, dispute of origin, and proof of premise. Transplantation 2007;84(09):1208–1209
22 Hernandez F, Andres AM, Encinas JL, et al. Preservation of the native spleen in multivisceral transplantation. Pediatr Transplant 2013;17(06):556–560