ORGAN-SPECIFIC INTERVENTIONS in INTESTINAL TRANSPLANTATION

Guido Trentadue

Propositions that accompany the thesis "Organ-Specific Interventions in Intestinal Transplantation" by Guido Trentadue, MD.

(1) Experimental evidence is now strong enough to study the effects of luminal preservation in real life procurement procedures. – *This thesis, chapter 1.*

(2) Brain death negatively influences the outcome of transplantation and should therefore be included in all models of intestinal preservation. – *This thesis, chapter 2.*

(3) Intraluminal oxygen supply ameliorates ischemic damage of the intestinal mucosa. – *This thesis, chapter 3.*

(4) Different combinations of luminal and vascular preservation solutions can give more insight into whether there is real benefit to be taken from luminal preservation. – *This thesis, chapter 4.*

(5) Directly intervening gut-homing immune cells is potentially beneficious for all intestinal transplant patients. – *This thesis, chapters 5, 6 and 7.*

(6) Molecular test panels that analyse mucosal biopsies should be developed for monitoring graft health while we wait for cheaper sequencing methods to arrive. – *This thesis, discussion.*

(7) It will be possible to consider the progresses of medicine and bioengineering as real achievements for humanity when everyone has access to their benefits, and they stop being a privilege for the minorities. – *René Favaloro, 1999.*

(8) A barrier was built between clinical medicine and science in Argentina that must be tore down to give way to an improved care for the society and the patient.

(9) The ego must be eradicated from Science and scientific publications.

Proposiciones que acompañan a la tesis "Intervenciones organoespecíficas en trasplante intestinal" por Guido Trentadue, MD.

(1) La evidencia experimental es ahora lo suficientemente fuerte para estudiar los efectos de la preservación luminal en procedimientos de ablación en la vida real. – *Esta tesis, capítulo 1.*

(2) La muerte encefálica influencia negativamente el porvenir del trasplante y debe ser incluida en todos los modelos de preservación intestinal. – *Esta tesis, capítulo 2.*

(3) El suministro de oxígeno luminal mejora el daño producido por isquemia en la mucosa intestinal. – *Esta tesis, capítulo 3.*

(4) Diferentes combinaciones de soluciones de preservación luminal y vascular pueden dar mejor información sobre la verdadera existencia de beneficios brindados por preservación luminal. – *Esta tesis, capítulo 4.*

(5) Intervenir directamente a las células que vuelven al blanco intestinal es potencialmente beneficioso para todos los pacientes con trasplante de intestino. *Esta tesis, capítulos 5, 6 y 7.*

(6) Paneles de análisis moleculares que investigan biopsias de la mucosa intestinal deberían ser desarrolladas para monitorear la salud del injerto mientras se espera por el arribo de métodos de secuenciación más baratos. – *Esta tesis, Discusión.*

(7) Los progresos de la medicina y de la bioingeniería podrán considerarse verdaderos logros para la humanidad cuando todas las personas tengan acceso a sus beneficios y dejen de ser un privilegio para las minorías. – *René Favaloro, 1999.*

(8) Se construyó una pared entre la medicina clínica y la ciencia en Argentina que tiene que ser derribada para dar paso a un cuidado mejorado de la sociedad y del paciente.

(9) El ego tiene que ser erradicado de la Ciencia y las publicaciones científicas.

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Monty Kaplan has kindly lent his photograph (Untitled, taken in Buenos Aires in 2016) for the cover of this book.

Lynette de Vries has kindly lent her photographic skills for the author's photograph.

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university of groningen

Organ-Specific Interventions in Intestinal Transplantation

Towards a longer survival of the intestinal graft

PhD thesis

to obtain the degree of PhD at the
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the decision by the College of Deans.

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"It's not a book, it's research!"

Neil Breen.

Improving the success of intestinal transplantation: studies on luminal preservation and treatment of acute and chronic rejection.

List of abbreviations.

ACR	Acute cellular rejection.
CAE	Chronic allograft enteropathy.
CIT	Cold ischaemia time.
DBD	Donation after brain death.
ITx	Intestinal transplant(ation).
TPN	Total parenteral nutrition.

Background.

Intestinal transplantation (ITx) has emerged as a life-saving treatment for patients with (chronic) intestinal failure that do not respond to replacement therapy such as total parenteral nutrition (TPN) or surgical procedures such as the serial transverse enteroplasty or longitudinal lengthening techniques[9].

Although effective, TPN impairs the patient's quality of life[11] and is a burden to the finances of the healthcare system[12,13]. Later-Nobel prize laureate Alex Carrel first thought of ITx in 1902. However, the first procedures in humans were only performed in the early 1960s, and yielded a maximum patient survival period of 76 days[22]. In 1987, Thomas Starzl reported about the first patient with extended survival (192 days)[28]. With tacrolimus becoming a part of the armoury to fight graft rejection in the early nineties, ITx became increasingly more common. Since then, transplantation, graft and patient survival rates have increased, with the former reaching a peak in the early part of the last decade[31]. Nevertheless, because of several improvements in TPN, ITx, although cost-effective, became a less attractive treatment, so the rate of procedures decreased. In the meantime, the survival rates plateaued.

This thesis aims to discover new treatments directed exclusively at the organ in different stages of the transplantation procedure. This is necessary because of a limited ten-year graft and patient survival rates of 41% and 47%, respectively[32,33]. The luminal content of the intestine continuously challenges the intestinal mucosa. That, in addition to strong immunosuppressive treatments, can lead to serious infections. The mucosa also harbours, unlike other solid organs, an extreme lymphoid tissue load, making it prone to inflammatory reactions such as what occurs during rejection. Contrary to current standards, necessary improvement of both graft and patient survival rates warrants a more organ-specific approach during preservation and for the prevention and treatment of rejection.

The transplantation procedure.

Although ITx can be performed with living donation, most patients receive the intestine following donation after brain death (DBD). The small intestine (some transplant centres include the large intestine, too[34]) can be transplanted alone or accompanied by other abdominal organs such as the liver, pancreas, stomach and kidney[35]. Several criteria must be met to be able to accept the donor's intestine, both from the donor and from the recipient's side. Many of these criteria are common to all organs and been published[36] and updated[37] over the years. Our

research group also evaluated the state of the organ at procurement and found an association between the optimal microscopic and molecular state of the graft and the fulfilment of the criteria[39]. In DBD lies the first difficulty for the intestinal graft, because the pathophysiological events that are at play during brain death negatively affect the bowel: the intestinal barrier begins to break down slowly creating an inflammatory microenvironment in the lamina propria from here on[40].

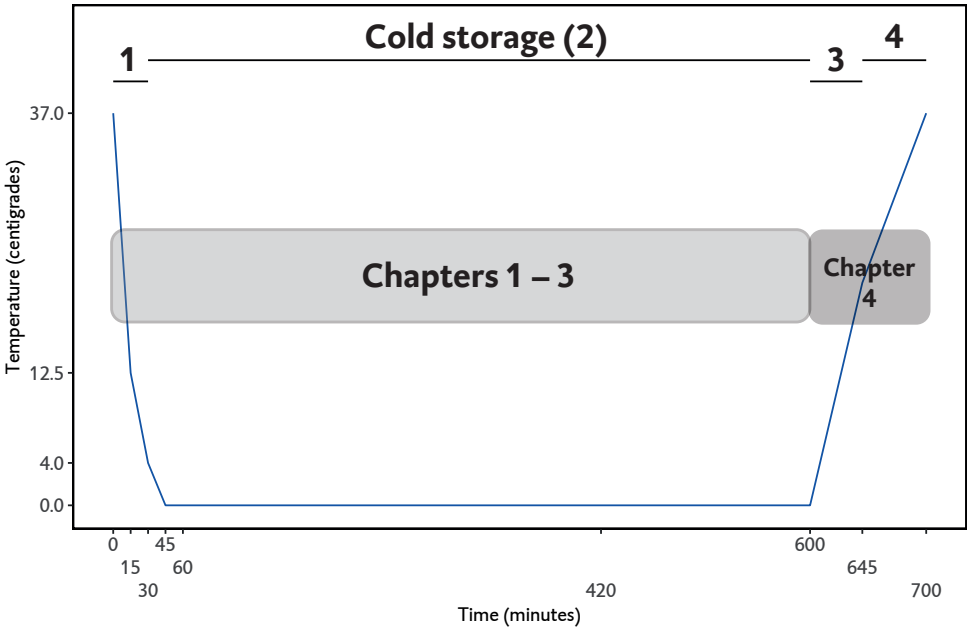

Figure I. **A diagram of the phases of the transplantation procedure**. (1) Temperature transition period, during vascular flush. (2) Cold storage normally lasts around six to nine hours. (3) Revascularisation. (4) Reperfusion. The warm ischaemia time is composed of (3) and (4). Section A of this thesis will cover these phases as seen on the graph.

The transplantation procedure is complex and is comprised of several phases (Figure I) that can also affect the organ in a negative manner. Vascular perfusion with a standard preservation solution at ice-cold temperatures defines the first phase in organ preservation. This period can take approximately fifteen to thirty minutes depending on the solution used. The longer this period lasts, the worse it is for the organ, as metabolic demands can still be high in relation to the diminishing supply from the disappearing blood. The cold ischaemia time (CIT) thus commences as the cold preservation solution enters the body. At this point, the abdominal organs are covered with ice-cold saline and preservation solutions to maintain the hypothermia while each organ is dissected. Here, the intestine is placed directly into a bag prefilled with ice-

cold preservation solution, closed, and placed in the organ box filled with ice. Now, the healthcare team has about ten hours to implant the graft into the recipient before it becomes irreversibly damaged[41]. Again, this period becomes more deleterious the longer it lasts, as damage seems to evolve in a time-dependent manner[42]. The moment of implantation (anastomosis phase) is of importance as the organ warms up and its temperature shift generates damage. This phase lasts about 45 minutes if there are no complications. After completion of the vascular anastomosis, reperfusion starts and a massive flow of recipient blood encounters the donor's intestine, pushing and redistributing cytokines and potentially toxic metabolites that were generated since brain death and during CIT into the rest of the graft and eventually out of it. Ischaemia-reperfusion injury occurs at this time, leaving the organ affected in ways invisible to the naked eye that have the potential to influence the three most common causes of graft failure: acute and chronic rejection, and infections[32].

The inflammatory process.

The inflammatory microenvironment created during these early phases in ITx is thought to have an impact on short and long-term graft health. Additionally, a very important mediator of the injury is the (re)entry of circulating inflammatory cells into the graft's mucosa. The interplay between damage-associated molecular patterns generated during brain death and ischaemia, pathogen-associated molecular patterns introduced into the mucosa during the breakdown of the barrier during preservation, and actions performed by naïve and primed immune cells not only affect the graft during moments of reperfusion but can be long lasting[1]. Depending on the severity of the inflammatory responses, the graft can suffer damage immediately (hyper acute rejection), within a few weeks (acute rejection), or for up to many years post-transplantation (chronic rejection).

Recently, new biological treatments specifically targeting the intestine for inflammatory bowel diseases have created the opportunity to investigate whether such approaches are appropriate to manage the immune system in ITx. Many processes in rejection involve some sort of contact between inflammatory cells and the vessel walls of the graft. Blockade of gut-homing reactive T-cells and other inflammatory cells re-entering the mucosa may be used to prevent major complications.

Acute cellular rejection (ACR) is mediated mainly by activated T-cells. The working theory of how gut-homing blocking could work in these cases is by minimising the inflammatory burden

by preventing these cells to reach their destination[43]. Certain aspects of this mechanism have been questioned by observations that suggests that vedolizumab does not prevent the interaction between the integrin (on activated immunity cells) and its receptor (on the gut mucosal endothelium), but by manipulating naïve responses by shifting macrophage types present in the lamina propria[44].

Chronic rejection of the intestinal graft (chronic allograft enteropathy, CAE) is characterised by a slow development of ischaemic lesions in the mucosa that appear due to the gradual thickening of the mid to large-sized vessel walls in the graft, as well as other characteristic effects in the outer layers of the intestine[45]. Some characteristics of this disease are like that of atherosclerosis, and there is evidence pointing towards an increased expression of the integrin α4β7, targeted by vedolizumab[46]. Although not yet common knowledge, this aspect of the disease could be of interest to prevent irreversible graft failure at this stage, which nowadays is solved only with graft enterotomy.

Aims and outline of thesis.

The stagnation of the worldwide number of ITx procedures does not mean that its benefits should be questioned. This thesis aims to build upon current knowledge in the field of ITx and explores different methods to improve its survival rates, primarily by using organ-specific methods. More specifically, it investigates the effects of luminal preservation of the intestine during cold storage and the final warm ischaemia time with luminal treatments. The effect of brain death, a generally ignored injury mechanism in experimental models, has been included in some of the studies. Finally, it proposes new therapies to manage acute cellular and chronic rejection of the intestinal graft by using the gut-homing blocker vedolizumab.

SECTION A of this thesis will thus focus on luminal preservation of the intestine (Figure I). **Chapter 1** reviews the experimental methods and results of luminal preservation experiments, recognising what is missing to fill those voids in future investigations. **Chapter 2** is an experimental study on the effects of luminal preservation in brain dead rats with two different (and previously successful) preservation solutions. **Chapter 3** is an application of luminal preservation in human donors performed between 2015 and 2020 in The Netherlands and Belgium, in a setup completely adapted to the clinical procurement procedure. **Chapter 4** uses a pig model to investigate the effects of luminal preservation during warm ischaemia in a setup that assimilates certain aspects of the engraftment procedure.

SECTION B focuses on the aftermath of the transplantation by investigating new ways to manage graft rejection. **Chapter 5** reviews the pathophysiology of the immune mechanisms at play during graft tolerance and rejection. Following, this thesis describes in-depth two case reports of successful treatment of both acute cellular rejection (**Chapter 6**) and chronic allograft enteropathy (**Chapter 7**) using vedolizumab. In **Chapter 8**, sequential biopsies taken from these two patients before and during treatment are analysed by RNA sequencing to discover transcriptional patterns associated with the success of the treatment.

SECTION A.
LUMINAL PRESERVATION.

"(…) My work, [was pursued] chiefly from a craving after knowledge (...). And therewithal, whenever I found out anything remarkable, I have thought it my duty to put down my discovery on paper, so that all ingenious people might be informed thereof."

Antony van Leeuwenhoek.