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PRESERVATION OF THE SMALL INTESTINE FOR TRANSPLANTATION

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August, 1995

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

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ABSTRACT

Transplantation of the small intestine is technically feasible, and the only potentially curative method for patients with short gut syndrome. However this procedure is still infrequent, partially because there is still no reliable method to preserve the small bowel (SB) for a reasonable period of time between removal from the donor and transplantation. In search of a suitable medium for SB preservation, we evaluated different solutions which have been successfully used for preservation of other human organs (Eurocollins (EC), University of Wisconsin (UW) and lactated Ringer's (LR) ) and tried to improve their effectiveness by adding superoxide dismutase and catalase or verapamil. The adequacy of preservation was assessed by evaluating the physiological properties of the intestine in vitro, using either the rat syngeneic model of intestinal transplantation, or human intestine obtained from organ donors.

LR is a simple, inexpensive and universally available solution, and when supplemented with verapamil, it was as effective as the more complex EC and UW as a protectant against ischemic damage during cold storage of rat ileum. Studies on human intestine validated the rat as an experimental model since the relative effectiveness of the different solutions was similar, however, the human bowel appeared more vulnerable to ischemic and mechanical damage. The results indicate that creation of an effective preservation solution for the small intestine should be possible through appropriate modification of currently available preparations.
La transplantation (TX) de l'intestin grêle est techniquement possible et, en soi, est le seul traitement curatif chez les patients souffrant du syndrome d'intestin court. Néanmoins cette méthode n'est pas souvent utilisée, en partie, à cause de l'absence d'une conservation adéquate avant la TX. Dans le but de trouver un milieu approprié pour l'intestin, différentes solutions (Eurocollins "EC", University of Wisconsin "UW" et le lactated Ringer's "LR"), utilisées avec succès dans la conservation d'autres organes humains, ont été évaluées puis améliorées par l'ajout de "vérapamil ou de superoxide dismutase + catalase". L'efficacité de la conservation a été testée par l'évaluation, in vitro, des propriétés physiologiques d'intestin de rat, avant et après la transplantation (animaux identiques), et d'intestin humain obtenu de donneurs d'organes.

Après l'ajout du vérapamil, Le LR un milieu simple, non dispendieux et facile d'accès, était tout aussi efficace que des solutions complexes comme le EC et le UW. Les études sur l'intestin humain soutiennent que le rat est un modèle valable de transplantation expérimentale. Cependant, l'intestin humain semble plus vulnérable aux dommages ischémiques et physiologiques. Ces résultats indiquent que la création d'un milieu de conservation efficace pour l'intestin grêle devrait être possible avec la modification des solutions existantes.
ABBREVIATIONS

CCB: Calcium channel blockers
CR: Contractile response
CsA: Cyclosporine
EC: Eurocollins solution
ECV: Eurocollins solution + verapamil
ED50: Dose producing 50% of the maximal effect
Exc: Excitatory innervation
FRS: Free radical scavengers
GVIIR: Graft-versus-host response
h: Hour
Inh: Inhibitory innervation
IVC: Inferior vena cava
LR: Lactated Ringer’s solution
LRV: Lactated Ringer’s solution + verapamil
NANC: Non-adrenergic non-cholinergic
NO: Nitric oxide
PBS: Phosphate buffer sucrose solution
pD2: Negative logarithm of ED50
PV: Preservation
SB: Small bowel
SBP: Small bowel preservation
SBT: Small bowel transplantation
S+C: Superoxide dismutase and catalase
SGS: Short gut syndrome
SMA: Spontaneous myogenic activity
TPN: Total parental nutrition
TX: Transplantation
UW: University of Wisconsin solution
UWV: University of Wisconsin solution + verapamil
INTRODUCTION

General description of the project.

Small bowel transplantation (SBT) is slowly gaining acceptance as the optimal treatment for people with short gut syndrome (SGS). Although Lillehei first demonstrated the feasibility of SBT in 1959, there are several problems associated with this procedure that remain to be solved; the most immediate of which is the lack of a reliable method to preserve the intestine during the interval between removal from the donor and transplantation (TX) into the recipient. SBT is therefore a complex procedure because the inability to store the organ necessitates two surgical teams: one for the donor and another for the recipient. Invariably, the donor and recipient operations have been performed during the night when operating rooms are available in the donor hospital. Extending the preservation time for the SB to 30 h would have the same impact on its TX that it did on kidney and liver TX i.e: allow more time for recipient preparation and tissue typing, increase organ availability, decrease organ wastage, increase organ sharing, and reduce cost. A technique to anticipate the extent to which the donor organ could recover function upon implantation would also assist in maximizing graft and patient survival.

The work reported in this thesis was designed to further the development of a simple and effective method for small bowel preservation (SBP) which would prolong graft storage time and possibly improve graft function. This goal includes the assessment of a method to estimate the potential survival of the donor intestine.
Reasons for intestinal transplantation.

SBT is the only curative treatment for people with SGS which is characterized by malnutrition, weight loss, steatorrhea, and acidic diarrhea resulting from absence, disease, or resection of extensive portions of the intestine. Massive resection of the small bowel in children most commonly results from necrotizing enterocolitis, midgut volvulus, extensive aganglionosis, hypoganglionosis, atresia, motility disorders and strangulated abdominal wall defects. In the adult population, massive bowel resection most commonly results from mesenteric vasculopathy, inflammatory bowel disease and trauma. Patients with SGS present an important clinical problem that necessitates long-term artificial support of nutrition in the form of total parenteral nutrition (TPN). Most TPN patients experience various problems such as catheter-related septicemia, hepatic abnormalities and psychosocial problems. The success of the technical aspects of SBT has brought new demands for transplantation in order to help these patients to achieve normal function.

Clinical progress in intestinal transplantation.

Despite the longer evolution compared to progress in other solid organ transplants, SBT is now a clinical reality. The first complete intestinal transplant was done at the University of Minnesota in 1967. Interest in clinical SBT waned with the advent of TPN in the early 1970s and the absence of success in the seven reported patients. In the 1980s, the growing awareness of the limitations of TPN and the ability to combat rejection with cyclosporine (CsA) revived interest in SBT.

Functional human intestinal transplant was first achieved in 1985 in Toronto by Cohen and
his group in a twenty-six year old female patient who had undergone a complete SB resection because of a large desmoid tumor secondary to Gardner's syndrome. She died ten days following surgery from probable CsA toxicity after having suffered from haemolytic anemia and the beginning of a rejection episode. In 1987 Starzl and his group\textsuperscript{13} reported the case of a three and a half year old black girl who had suffered perinatal volvulus and gangrene for which massive SB resection was required. The child developed progressive liver failure secondary to TPN and underwent multivisceral TX. Multifocal lymphoproliferative disease (LPD) was diagnosed on postoperative day 91 and the child died on day 192. Even with the use of CsA for clinical SBT, the results were disappointing.\textsuperscript{14,15} Most of the recipients lost their grafts as a result of rejection or uncontrollable infection; only one adult and one child were able to resume normal oral nutrition for a prolonged time.

It is only in recent years that successful SBT has been carried out in USA, Canada\textsuperscript{16,17} and Europe.\textsuperscript{18} Two refinements have allowed SBT to become a more successful option in the 1990s. First, a protocol has been developed for successful SB/liver TX using CsA.\textsuperscript{18} This operation has been particularly useful for the treatment of patients who have developed liver failure as a complication of long-term TPN. To date, more than 50 combined liver/SB transplants have been performed at eight centers (Birmingham, Boston, London-Ontario, Los Angeles, Madison, Omaha, Paris, and Pittsburgh) with a 70\% one year actuarial patient survival rate. The second major advance has been the discoveries of FK506, a new macrolide immunosuppressive agent, and prostaglandin E\textsubscript{1} which permit successful TX of the SB as an isolated graft.\textsuperscript{19,20,21} Using this protocol, more than 29 isolated SBTs have been performed at four of the previously cited centers (Cambridge, London-Ontario, Omaha, and Pittsburgh) with a 70\% 1-year actuarial graft survival
Isolated SBT is therefore feasible. Long-term survival with adequate graft function and normal growth can be achieved. Some patients with small bowel transplants for up to 2 years are leading relatively normal lives and feeding entirely by the enteral route.

One of the critical variables in any organ TX procedure is the interval between removal of the organ from the donor and the implantation into a suitable recipient. The longer the organ can be stored prior to implantation, the greater the chance for identification of a more suitable recipient at a distant location, and the less critical the transport time. At the present time, there is little information concerning the optimum method for PV of the human small intestine.

Experimental studies on organ preservation.

In the late 1960s two important studies demonstrated the safe preservation of kidneys: for thirty hours by cold storage and as long as seventy two hours by continuous perfusion (EC, perfusate with adenosine phosphate). These two studies changed clinical kidney TX from an emergency procedure to a semielective procedure. Many investigators have tested other cold-storage solutions and some have claimed successful preservation of kidneys for 48 or 72 hours using UW and EC with human atrial natriuretic peptide. The introduction of Cyclosporine (CsA) for immunosuppression during the 1980s revived interest in transplantation of other organs, but preservation methods that are successful for other organs are not effective for small bowel preservation.
Experimental studies on intestinal transplantation.

SBT was first tested as a treatment for SGS by Carrel\textsuperscript{31} in the early 20th century, using isolated loops of jejunum transplanted into the neck of dogs. But the possibility of SBT was taken seriously after Lillehei and collaborators\textsuperscript{1} demonstrated the technical feasibility while investigating the effects of ischemia on abdominal organs. They used a dog model wherein they found that cooling and perfusion with heparinized saline would reliably allow preservation of the SB for 4 h, and that preserved bowel could be reimplanted and would function indefinitely as an autograft.\textsuperscript{11} Many models have since been used in the study of SBT. Pigs have been studied as they closely resemble the human in physiology and have a more defined genetic background than the dog. The large mammal models have been utilized more for preclinical evaluation. Monchick and Russell\textsuperscript{32} in 1971 were the first to describe the rat model. The rat model, even though technically difficult, is the one most commonly used; it has economic advantages and serves as the standard for initial investigations.

Many techniques for preservation of the SB have been tried experimentally, ranging from simple hypothermic storage in a hyperbaric oxygen chamber by Manax et al. in 1965, to pulsatile normothermic perfusion by Hohen-Leitener and Senior in 1969. Intravascular flushing with a cold (4°C) crystalloid solution followed by cold storage appears to be technically easy and economical (Ricour et al. 1981), and it preserves kidneys for up to 24 h (Mozes et al., in 1985). The rationale for lowering temperature in organ preservation is to reduce the metabolism of the organ, reduce the energy required, and reduce the accumulation of potentially toxic metabolites. Even a brief exposure to warm ischemia results in a decrease in organ function,
and after about one hour of warm ischemia most organs are irreversibly damaged. The rapid induction of cooling is, therefore, an essential factor in obtaining successful SBP.

Currently available preservation solutions. UW solution was developed by Belzer and Southard at the university of Wisconsin in 1988, and is effective in preserving kidney and pancreas for up to 72 hours and liver for 48 hours, and it is being used successfully in human liver transplantation. The composition of UW solution is designed to imitate intracellular fluid. UW, which contains lactobionate as an anion and raffinose as an uncharged impermeant, has its effect in retarding cell swelling which occurs during ischemic cold storage. EC, (another solution based on intracellular fluid, and developed by Collins et al in 1969), was the agent most commonly used in clinical organ preservation until recently. Phosphate buffered sucrose, a relatively new simple solution, and EC have a high concentration of impermeants (glucose and sucrose). They also contain a high concentration of phosphate, which may also play a role in successful kidney preservation. UW and EC are both high K⁺, low Na⁺ solutions. Their use is thought to prevent K⁺ leakage from stored cells and to maintain the normal intracellular milieu. Reduction of the leakage would decrease the activity of the Na⁺ -K⁺ ATPase pump and thus conserve high-energy phosphates. Thus the cell would expend less energy re-establishing the intracellular milieu after revascularization. Along with PBS, newer less complex solutions are reported to be adequate for the preservation of different organs; Cardiosol for the heart, hypertonic citrate and lactobionate solutions for the lung, Dalhousie solutions for the liver, Perfluorochemical (PFC) for the pancreas, and the Marshal-Ross solution of hypertonic citrate and histidine-tryptophan-ketoglutarate (HTK) for the kidney. There is a continual attempt to improve the existing preservation media by supplementing the
formulas with additional ingredients. The rationale for additives like superoxide dismutase and catalase (S+C) is based on the observation that intestinal mucosa is very sensitive to ischemic injury and it contains a high concentration of xanthine dehydrogenase, which after reperfusion reacts with the oxygen to generate oxygen metabolites. This burst of oxygen radicals damages tissue and causes necrosis. Superoxide dismutase effectively catalyzes the dismutation of superoxide radicals to hydrogen peroxide (H₂O₂) and oxygen. Metabolism of H₂O₂ to water is then accomplished by catalase. When administered to the organ or recipient, free radical scavengers (FRS) have been shown to result in much better function of transplanted rat small bowel following ischemia and reperfusion.

Verapamil, a calcium channel blocker (CCB) has been shown to improve myocardial preservation during cold cardioplegia, promote functional recovery in ischemic lung, protect kidneys during periods of both warm and cold ischemia, and protect liver from ischemic damage during cold storage. Alteration in intracellular calcium homeostasis plays a central role in the pathogenesis of tissue injury in ischemia. Calcium influx during ischemia causes elevation of intracellular free calcium which triggers a number of cellular processes, such as phospholipase activation, that ultimately result in damage to plasma membranes and mitochondria and eventually in cell death.
Goals of the project.

The present experiments were designed to find and/or improve a simple solution for SBP, to evaluate and compare complex preservation solutions which may be specifically beneficial to small bowel, and to elucidate the effectiveness of free radical scavengers\(^\text{44,45}\) and calcium channel blockers\(^\text{40}\) as additives to the preservation medium. Rat and human tissue samples from the ileum were studied using a previously developed screening procedure for preservation solutions. The physiological function of SB samples is an excellent indicator of ultimate graft viability and a useful and sensitive tool for monitoring graft function.\(^\text{47,48,49}\) Initial tests were conducted on the rat SB stored in different solutions, for different periods of time, prior to transplantation. Subsequent studies were performed on transplanted syngeneic rat bowel removed after a minimum survival period of two weeks. Solutions which resulted in successful rat survival with optimum graft function after implantation were further tested on human ileum. The experiments on human tissue provide essential preclinical data, and, also serve to further evaluate the usefulness of the rat model for subsequent studies.

Anatomy of the small intestine.

The small intestine is the longest portion of the intestinal tract; it includes the duodenum, the jejunum, and the ileum to the ileocecal valve. The duodenum-jejunum is arbitrarily defined as the upper two-fifths of the small intestine and the ileum as the remaining three-fifths. The mesentery of the small intestine is fixed to the left of the second lumbar vertebra and runs downward and to the right to the level of the right sacroiliac joint. The ileum is supplied solely by the superior mesenteric artery, which is why so much of the SB is infarcted upon obstruction
of this blood vessel. As the primary function of the SB is absorption of fluid, electrolytes, and nutrients, a large absorptive surface area is necessary. The small intestine provides for this large surface in three ways: 1) The adult small intestine is approximately 22 feet (657 cm) in length. Its surface area is amplified 3-fold by the intestinal folds, the plicae conniventes. 2) The mucosa is made up of invaginating finger-like projections into the lumen, the villi, an architectural arrangement that increases the absorptive cell lining. 3) The villi have multiple invaginating fingerlike projections of the plasma membrane on their luminal side, the microvilli or the brush border membrane that further amplify the absorptive surface by another 20-fold. Together, the intestinal folds, villi, and microvilli increase the absorptive surface area of a simple tube by 600-fold. In an adult human of average weight, about 9 litres of fluid (2 of oral intake and 7 litres of endogenous isotonic secretion) enter the upper small intestine per day. The majority of this total volume is absorbed in the SB; only about 1-1.5 litres enters the colon. Thus the small intestine absorbs approximately 7.5 litres of isotonic fluid in addition to ingested nutrients.

The wall of the small intestine is made of the serosa, the muscularis, the submucosa, and the mucosa. A cross section of the small intestine is illustrated in figure 1. Outermost is the serosa, made of a single layer of mesothelial cells overlying some connective tissue. Underlying the serosa is the muscle layer consisting of two components oriented in longitudinal and circular directions. The submucosa is primarily dense connective tissue that contains many other elements critical to the function of the small intestine: (1) elaborate vasculature, with arterioles, plus venous, and lymphatic drainage systems; (2) leucocytes (lymphocytes, macrophages, and eosinophils), mast cells and fibroblasts that release substances (i.e. serotonin, histamine,
prostaglandins) affecting the absorptive and secretory function of the SB; (3) a complex network of ganglion cells and nerve fibers that form the submucosal plexus which interacts with the autonomic nervous system; and finally (4) the submucosa of the duodenum also contains elaborately branched glandular structures, Brunner’s glands, which are most prominent in the proximal duodenum and become sparse toward the jejunum, and which secrete mucus and bicarbonate, the latter to neutralize the acid pouring down from the stomach.

The innermost layer of the wall, the mucosa, contains three subdivisions: (1) the epithelium, (2) the lamina propria, the basement membrane the epithelial cells rest on, and (3) a layer of smooth muscle (muscularis mucosa). The epithelium is composed of an enormous number of small, fingerlike projections into the lumen. The projections, which are a millimeter or so long, are called villi. At the feet of the villi are the openings of tubelike crypts of Lieberkühn. The lumenal surface appears furry because of the villi. Inside each villus there are lymphatic capillaries called lacteals. The lacteals and blood capillaries provide the two routes by which nutrients move from the vicinity of the intestine into the general circulation. Individual villi are also capable of motion due to the presence of strands of smooth muscle within the submucosa. The motion of villi is greatly increased when food is present. The structure of microvilli suggests that they may also be capable of motion. Each microvillus contains 20 to 30 actin microfilaments that extend some distance into the apical cytoplasm of the epithelial cells, a region referred to as the terminal web. Actin is involved in muscle contraction and may make movement and extension of the microvilli possible. The movement of villi and microvilli helps reduce the effect of the unstirred layer of fluid immediately adjacent to the apical surface of the
cells. Within the unstirred layer, solute movement is slow because it occurs primarily by diffusion rather than bulk flow.

The lamina propria is made of smooth muscle, collagen and elastin fibers, fibroblasts, and leukocytes. The cells of the lamina propria play an important role in the normal functioning of the intestine. The leukocytes are important in combating bacteria and in the processing and recognition of antigens. Moreover, these cells are important in the pathogenesis of inflammatory bowel disease (IBD): increased in IBD, they release soluble mediators called immune-inflammatory mediators (i.e., interleukins, prostaglandins, serotonin) that produce diarrhea both by inhibiting absorption and by stimulating secretion in the SB. The muscularis mucosa is a thin layer of smooth muscle that thrusts the epithelium into permanent, visible ridges called the plicae circularis or Kerckring's folds.

**Intestinal smooth muscle, vasculature and nerves.**

The smooth muscle cells of the small intestine are 50 to 100 microns long and 2 to 5 microns wide. Smooth muscle action potentials have a smaller amplitude and last longer (10 to 20 milliseconds) than those in neurons but last less than cardiac action potentials. Smooth muscle cells are usually arranged in sheets which are oriented longitudinally or circularly. A dual orientation is needed to generate peristaltic and segmentation movements. Smooth muscle cells can be excited by action potentials from other cells or can show an intrinsic pattern of periodic depolarizations called *pacemaker activity*. The pacemaker activity takes the form of slow waves of depolarization followed by repolarization, and is found in most parts of the GI tract. Each slow wave lasts 3 to 20 seconds, based on the location. Slow waves comprise the basic electrical rhythm that regulates the frequency and progression of peristaltic waves. Slow wave
depolarization maintains a level of tonic tension in smooth muscle, but this depolarization by itself is not large enough to cause a measurable contraction. Under certain conditions, however, a slow wave may exceed the threshold for action potential generation, in which case one or more action potentials occur during the wave. These action potentials initiate smooth muscle contraction. Smooth muscle contracts slowly, and the force produced depends on the number of action potentials occurring at the peak of the slow wave. In the interval between slow wave-initiated bursts of action potentials, smooth muscle tension is usually above zero, termed basal tone.

The jejunum and ileum are supplied by the superior mesenteric artery, the intestinal branches of which, having reached the attached border of the bowel, run between the serous and muscular coats with frequent diversions to the free border, where they also anastomose with other branches running around the opposite surface of the gut. From these vessels numerous branches are given off that pierce the muscular coat, supplying it and forming an intricate plexus in the submucous tissue. From this plexus minute vessels pass to the glands and villi of the mucous membrane. The veins have a parallel course and arrangement similar to that of the arteries.

The lymphatics of the small intestine (lacteals) are ordered in two sets, those of the mucous membrane and those of the muscular coat. The lymphatics of the villi commence in these structures in the manner described above. They form an intricate plexus in the mucous and submucous tissue and are joined by the lymphatics from the lymph spaces at the bases of the solitary lymph nodes; from this they pass to larger vessels at the mesenteric border of the intestine. The lymphatics of the muscular coat are located to a great extent between the two layers of muscular fibers, where they form a close plexus; throughout their course they
communicate freely with the lymphatics from the mucous membrane, and empty in the same way as these into the origins of the lacteal vessels at the attached border of the gut.

The nerves of the small intestine are from the plexuses of autonomic nerves around the superior mesenteric artery. They serve as cranial parasympathetic fibers of the vagus and postganglionic sympathetic fibers from the celiac plexus. From this source they run to the myenteric plexus (Auerbach’s plexus) of nerves and ganglia located between the circular and longitudinal muscular fibers, from which the nervous branches are distributed to the muscular coats of the intestine. From this, a secondary plexus, the plexus of the submucosa (Meissner’s plexus) is derived, formed by branches that have entered the circular muscular fibers. This plexus lies in the submucous coat of the intestine; it also holds ganglia from which nerve fibers pass to the muscularis mucosae and to the mucous membrane. The nerve bundles of the submucous plexus are smaller than those of the myenteric plexus.

**Physiology of the small intestine.**

The small bowel is the major site for both digestion and absorption of food. To survive, one must at least eat and drink. It is the function of the small intestine to digest and assimilate the numerous and in some cases, the excessive, amounts of food and liquid taken in. For digestion, carbohydrates, fat, and protein must go through reduction of particle size. The grinding action of the mouth and the stomach is primarily involved in the reduction of food to less than 1 mm size particles. The process of digestion that starts in the stomach and is well underway in the duodenum is completed throughout the jejunum and ileum. Absorption occurs at the brush
border membrane of the villus cells, while digestion takes place throughout the intestinal lumen as well at the surface of the epithelial cells.

The motor activity of the SB assists the primary function of absorption of nutrients by mixing and propelling the chyme inexorably toward the anus. After a meal has been eaten, the material is moved along in the intestine by slow wave activity that originates in the interstitial cells of Cajal at the junction of the two layers of the muscularis externa. The frequency of the slow waves is faster in the duodenum (12 cycles per minute) than in the ileum (8 cycles per minute). The increased frequency of slow waves in the proximal bowel helps propel the chyme to the distal SB, thus making the frequency gradient of slow waves control, at least in part, the aboral movement of chyme in the SB. Mixing is accomplished mostly by isolated stationary "standing ring" contractions of circular muscle involving a 1 to 2 cm segment of intestine or as a series of such contractions, referred to as rhythmic segmentation. Segmentation occurs only in the intestines, where it dominates peristaltic movements. Segmentation involves an alternating contraction and relaxation of the circular muscle layer in a restricted region of the intestine, with the segmentation site moving from one place to another without displacing the intestinal contents very far in either direction. As is true for the peristaltic wave of contraction, segmentation is initiated by the intrinsic depolarizations of smooth muscle cells, but the coordination of the circular and longitudinal muscle layers requires the enteric nervous system to act as a "visceral brain" for the SB.

The mucosal layer contains the endings of sensory cells specialized for chemoreception and mechanoreception. The cell bodies of these receptor cells are in the submucosal plexus. Enteric interneurons transmit information between the submucosal plexus and the myenteric plexus.
Effector neurons in the myenteric plexus respond to the sensory signals by initiating local contractile or secretory responses. Some myenteric motor neurons innervate the longitudinal and circular muscle layers of the muscularis externa and the muscularis mucosa, and other myenteric neurons synapse with the neurons in the submucosal plexus that control secretory cells. The enteric nervous system, fully capable of initiating and sustaining a postprandial motor response, is also influenced by inputs from the autonomic nervous system. Postganglionic parasympathetic fibers enter the myenteric plexus and activate motor neurons or interneurons to influence contraction or secretion.

Both the central nervous system and the intrinsic nervous system are involved in the control of SB motility. Sympathetic stimulation of the gut decreases SB contractions whereas parasympathetic stimulation increases them. This extrinsic input to the small intestine acts both directly on the smooth muscle and also on the intrinsic nervous system.

During the interdigestive period a long absence of motor activity (phase 1) is followed by random activity (phase 2) that is followed by intense motor activity that begins in the stomach and migrates down the intestine (phase 3). This phase 3 activity, the migrating motor complex (MMC), occurs every $1\frac{1}{2}$ h and is primarily responsible for clearing the gut to prevent stagnation and bacterial overgrowth. The intrinsic nervous system rather than the central system initiates MMC activity, although the CNS may modulate it. A variety of gut peptides (i.e., somatostatin, substance P, histamine, pancreatic polypeptide) can also initiate MMC activity. Feeding, insulin, cholecystokinin, and gastrin block MMC activity. Although coordinated intestinal motor activity may occur in the absence of extrinsic autonomic innervation, this system is important in modifying motor activity in relationship to other physiological responses.
within the body. (fig. 1a) In dogs, extrinsically denervated jejunum is capable of responding to solid and liquid meals in relatively normal fashion. Recently Kiyochi and collaborators demonstrated extrinsic sympathetic reinnervation of an implanted graft after three weeks. Unfortunately the structure and function of the extrinsic system are still not completely understood.

Abnormalities in SB motor activity can result in constipation, diarrhea, malabsorption, or pseudoobstruction. For example, the increased slow wave frequency of hyperthyroidism can lead to diarrhea; on the other hand, decreased slow wave frequency seen in myxedema may be responsible for constipation. Absence of MMC is associated with small intestinal stasis and such complications as bacterial overgrowth and malabsorption. Increased frequency of MMC in carcinoid syndrome, thyrotoxicosis or Crohn’s disease may also lead to diarrhea.

As motility patterns represent the final outcome of a coordinated interaction between contractile and relaxant events, communication between contractile and inhibitory neurones is of fundamental importance. Endogenous nitric oxide (NO) which derives from enzymatic conversion of L-arginine through NO-synthase, is involved in inhibitory non-adrenergic, non-cholinergic (NANC) neurotransmission in the gastrointestinal tract (GIT). Therefore NO represents an important mediator of various physiological and pathophysiological processes in the GIT. Both the $\alpha_2$-adrenoceptors and $K^+$ channels presynaptically modulate NO release from NANC nerves in the ileocolic junction, but the mechanisms of action underlying any effect of NO on intestinal fluid and electrolyte transport remain unclear.
MATERIALS AND METHODS

Animal model

Male Lewis rats weighing from 180 to 300 g, obtained from Charles River Canada (St-
Constant, Que) were used as donors and recipients in order to avoid the effects of both rejection
or GVHR (a syngeneic model). Conventional animal facilities accredited by the Canadian
Committee of Animal Care (CCAC) were used for housing the rats. All animals were fed with
rat chow and tap water ad libitum. Approval of the institutional animal ethics committee was
obtained and the guidelines of the CCAC were followed in order to carry out experiments. Both
the donor and the recipient animals were fasted for one day prior to surgery then anaesthetized
with pentobarbital 50 mg/kg intraperitoneally. The syngeneic rat model is excellent for the study
of SBP and TX. This model provides the advantages of low cost and relative resistance to
sepsis.

Surgery.

CONTROL AND DONOR SURGERY: During the entire procedure, all rats were kept on
a heating pad at its lowest setting. Sterile technique was observed throughout the procedure,
which was performed with the aid of a Zeiss OPMI-6 microscope. The abdomen was shaved,
swabbed with alcohol, and draped with sterile towels and a steri-drape (3M-1020) with the
central hole over the midline of the abdomen. A midline incision was made and the bowel
eviscerated. In control preparations the entire SB from the ligament of Treitz to the terminal
ileum was quickly extirpated and immediately placed in Krebs solution. The bowel lumen and
vasculature were gently flushed, respectively, with 10 and 5 ml of chilled Krebs solution, then the intestine was dissected for physiological experimentation. In transplant studies, a total colectomy was done and the entire small bowel from the ligament of Treitz to the terminal ileum was harvested with the accompanying superior mesenteric artery on a cuff of proximal aorta, along with the superior mesenteric vein as seen in figure 1b. Before isolation of the bowel vasculature, the animals were systemically heparinized (100-200 U), then the bowel lumen was flushed with 10 ml of a chilled 0.5% neomycin sulfate preservation solution. The SB and its vascular pedicle were then quickly resected and the arterial lumen was immediately perfused with 5 ml of the chilled heparinized storage solution (10 U/mL). The bowel samples were either immediately taken for physiological study or preserved in the storage solution for different periods of time.

RECIPIENT SURGERY: Similar preoperatory preparations described above for donors were carried out for the recipient animals. The abdomen was shaved and swabbed with alcohol. As with the donor, the draping procedure, including the steri-drape was accomplished. An illustration of graft implantation is seen in figure 1c. The abdomen was entered through a midline incision and a self-retaining retractor was inserted. The native bowel was eviscerated and retracted superiorly then the ligament of Treitz was placed under minimal tension and divided sharply, thus freeing the duodenum from the retroperitoneum and colon for a long enough segment to obtain access to the vascular structures without tension on the bowel itself. The aorta was separated from the inferior vena cava (IVC) by blunt dissection. The infrarenal aorta and IVC were isolated and cross-clamped both proximally and distally. A venotomy with donor SMV was made after removing an ellipse of tissue from the IVC, and the lumen between
the clamps was irrigated with heparinized saline solution. An arteriotomy with donor SMA was performed on the aorta and the lumen was also irrigated with heparinized saline solution. In both vessels continuous anastomosis was performed with 10-0 nylon suture (either 10-0 Ethilon BV75-4, Ethicon or 10-0 Dermafix TE-75, Davis-Geck). The clamps were removed and the reperfused bowel was inspected. Successful reperfusion was immediately visible as pink color intensified. Both the proximal and distal ends of the graft were brought out as Brook’s stomas on the right flank. (fig. 1d) The native bowel was repositioned in the abdominal cavity and the abdomen was closed with running 4-0 Vicryl on the fascia and 5-0 Vicryl on the skin. The animals were given a total of 10-15 ml of lactated Ringer’s IV during the surgical procedure. Once surgery was completed, the recipient rats were allowed to awaken in an incubator at 38°C before returning to their cages. All rats surviving two weeks after graft implantation were considered as survivors.

**Human model.**

The use of human gut was possible with the collaboration of Transplant Quebec and the use of such tissue was approved by the appropriate ethics committees of the participating hospitals. The only specimens examined were from those donors where proper consent for this donation was obtained. Sixteen bowel segments were harvested at different hospitals from different organ donors ranging in age from 9 to 49 years. Only intestines where the donor protocol did not include early ligation of the superior mesenteric artery were used. The bowel was extirpated at the end of the harvesting procedure. Bowel segments of approximately 25 cm were dissected with the superior mesenteric artery and superior mesenteric vein, and vessels of the mesenteric
border were ligated. Tissue samples from bowel freshly harvested (1 to 5 h) and flushed with UW were taken for physiological studies (control).

The lumen of the remaining segments was flushed with 100 ml chilled preservation solution (UW, EC or LR) containing 0.5% neomycin sulfate, and then stored for 24 h in a container with the corresponding chilled oxygenated preservation solution. Subsequent evaluations were performed every 24 h on the additional segments, and the remaining of responsive bowel segments were reoxygenated and returned to storage.

**Research design.**

Unlike the situation for other organs, almost no experimental studies have been done on human bowel preservation. The bowel is probably more vulnerable to ischemic damage than organs like the kidney as it contains a large neural component which must remain functional after transplantation. However, the experimental studies to date indicate it should be possible to preserve the intestine through cold storage if a suitable preservation medium is developed. The utilization of a simple preservation solution would provide maximal benefit for clinical transplantation of the intestine. With complex solutions modelled on intracellular fluid (high K⁺), reperfusion of the transplanted intestine may result in cardiac arrest, when the large quantity of K⁺ in the graft vasculature reaches the systemic circulation of the recipient. In this situation, a final flush of the donor vasculature with a low K⁺ solution would be required, but with this final step, there is a risk of damaging the endothelium.

The work reported in this study was therefore designed to develop a simple and specific preservation solution for the small intestine and/or to elucidate the effectiveness for SBP of some
solutions previously developed to preserve other organs. We also examined the effects of adding CCB and FRS to the preservation solutions, since administration of these agents to some organs or recipients has been shown to result in much better function following ischemia and reperfusion.\textsuperscript{39,40,66,67,68}

Rat and human tissue samples from the ileum were studied in vitro using a screening procedure based on physiological and pharmacological examinations of smooth muscle and nerves. Prior to TX, an initial test was conducted on the rat SB stored in different solutions for different periods of time. Using the syngeneic rat model, subsequent studies were performed on grafts removed after appropriate survival of the recipient. The most promising solutions in the rat studies were tested in human ileum using the same parameters to assess graft viability.

Four primary solutions (Phosphate Buffered Sucrose "PBS-140", Lactated Ringer’s "LR", University of Wisconsin "UW", Eurocollins "EC") were evaluated in this study (see table 1 for composition of solutions). The effectiveness of some solutions with the added free radical scavengers\textsuperscript{42,45} superoxide dismutase and catalase or the calcium channel blocker\textsuperscript{40,42} verapamil were also assessed. Verapamil, 25 $\mu$g/ml, was directly added to the preservation solutions. The FRS superoxide dismutase plus catalase were used in a total dose of 5000 IU each, and administered with 5 ml of the chilled solution via superior mesenteric artery perfusion quickly after extirpation and again before implantation of the graft.

For physiological studies, both rat and human samples were mounted in isolated tissue baths (figure 1e,1f). The equipment used has been specially designed to handle tissue as small as 1 mm internal diameter (fig. 1e). Examination of multiple samples was easily conducted using up to 14 baths simultaneously. Each bath (20 ml) was filled with physiological solution, a
modified Krebs solution, bubbled with 95% oxygen and 5% carbon dioxide. The temperature of the solution in the baths was maintained at 37°C by a Haake heating pump (model FJ, Haake inc., Saddlebrook, NJ). Composition of the solution used in baths was: NaCl 118.2, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25.0, CaCl₂ 2.5, choline chloride 0.036, dextrose 10.0 and calcium EDTA 0.21 (all components are from Fisher Scientific Company, Canada, and values are in mmol/L). One extremity of the sample was attached with silk to a Grass force transducer (model FT03C; Grass Instruments, Quincy, MA), and the other extremity was fixed to the holder. The specimen was mounted with an initial tension of 1.0 g. Each bath accommodated parallel platinum electrodes connected to a Grass S48 stimulator for field stimulation. Electrical field stimuli (20 Hertz, 1 millisecond) lasting for 30 seconds were delivered every 4 minutes to each tissue. Tension was recorded on Beckman models RM, R511A and R611 ink-writing dynographs (Beckman instruments, Inc, Fullerton, CA) using model 9853A couplers and filter settings of DC to 100 Hertz. After adequate equilibration of graft tissue during exposure to intermittent transmural electrical field stimulation (TEFS) for approximately 1 h, the intrinsic tone of the specimen stabilized, and the experimental testing was begun. Tissue responses to TEFS and cumulative doses of the cholinergic agonist carbachol (10⁻⁷ to 10⁻³ mol/L) were examined. Muscle sensitivity to carbachol was quantified by determining the pD₂ value which was calculated by taking the negative log of the ED₅₀, or effective dose 50, from the cumulative dose-response curve. Larger values of pD₂ reflect a greater sensitivity to the drug.

Physiological and pharmacological properties, the predictors of graft viability, were thus evaluated via the following parameters: spontaneous myogenic activity (SMA), muscle contractility in response to the cholinergic agonist carbachol (CR), excitatory (Exe) and
inhibitory (Inh) innervation in response to electrical field stimulation. The physiological parameters were estimated by the percent of samples that showed activity. Previous studies in our laboratory on rat bowel transplantation demonstrated that these parameters were effective in determining which conditions maintain better viability of the graft for subsequent graft survivability after transplantation.

The statistical significance was tested using the one-way analysis of variance (ANOVA) to evaluate the preserved small bowel inhibitory activity when more than two solutions were compared, and using the student's t test to compare two solutions. A \( p < .05 \) was considered significant.
<table>
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<th>Names</th>
<th>PBS-140</th>
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<th>EC</th>
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<td>130</td>
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<td>10</td>
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<td>-</td>
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<td>6.7</td>
<td>7.4</td>
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Values in mM except where otherwise indicated.
PBS-140: Phosphate buffered sucrrose
LR: Lactated Ringer's
UW: University of Wisconsin
EC: Eurocollins
Figure 1. **ANATOMY OF THE SMALL INTESTINE**

1. Mesentery with vessels and nerves
2. Longitudinal muscle layer
3. Submucosa
4. Plicae circulares
5. Muscularis mucosae
6. Intestinal villi
7. Serosa
8. Myenteric plexus
9. Circular muscle layer
Figure 1a. INNERVATION OF THE SMALL BOWEL

Intrinsic innervation          Extrinsic innervation

VAGUS NERVE

SYMPATHETIC GANGLION
Figure 1b. GRAFT EXTIRPATION

SMA
PANCREAS
COLON
SMV
GRAFT
CÆCUM
Figure 1c. GRAFT IMPLANTATION

- GRAFT
- RENAL V.
- SMV
- SMA
- IVC
- AORTA
Diagram illustrating the experimental model. Grafts have been preserved in different solutions and periods of time prior to transplantation. After appropriate recipient survival the graft is then extirpated and compared with control (native bowel).
EXPERIMENTAL TECHNIQUE USED FOR MEASURING ISOMETRIC TENSION OF ISOLATED SMOOTH MUSCLE

Figure 1e.

- FORCE-TRANSUDER
- POLYGRAPH
- MODIFIED KREBS' SOLUTION
- 95%O₂ + 5%CO₂
- LONGITUDINAL STRIP
- CIRCULAR RING
- STIMULATOR
- ELECTRODE
- HOLDER
Figure 1f. PHOTOGRAPH OF TISSUE BATHS WITH TRANSDUCERS AND POLYGRAPH
CHAPTER 2

EFFECTIVENESS OF LACTATED RINGER’S, EUROCOLLINS AND UNIVERSITY OF WISCONSIN SOLUTIONS, ALONE OR WITH VERAPAMIL FOR RAT SMALL BOWEL PRESERVATION IN VITRO
EFFECTIVENESS OF LACTATED RINGER'S, EUROCOLLINS AND UNIVERSITY OF WISCONSIN SOLUTIONS, ALONE OR WITH VERAPAMIL FOR RAT SMALL BOWEL PRESERVATION IN VITRO

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Key words: Rat Ileum Preservation, LR+V, EC, UW, Graft Viability.
Successful experimental small bowel transplantation was first demonstrated by Lillehei and coworkers in 1959.\(^1\) Since then only a relatively small number of intestinal transplants have been attempted in humans. Despite advances in combatting rejection with cyclosporin, FK506, 15-deoxypergualin and other new immunosuppressive methods,\(^2,3,4,5\) intestinal preservation remains a major difficulty, and this limitation essentially requires the donor and recipient to be in the same institution. An effective and extended preservation period for the small intestine would both increase the donor pool and give more time for recipient preparation and tissue typing. In children with short gut syndrome, which commonly results from necrotizing enterocolitis, midgut volvulus, extensive aganglionosis, hypoganglionosis, atresia, motility disorders and strangulated abdominal wall defects,\(^6,7\) SBT is the only curative treatment.

Both the central nervous system (CNS) and the intrinsic nervous system (INS) are involved in the control of small bowel motility.\(^8\) Reinnervation by nerve endings of the resected extrinsic sympathetic system has been demonstrated after transplantation.\(^9,10\) The neurons of the INS are more vulnerable, as the cell bodies are located within the graft itself, and effective preservation of graft INS is essential for successful small bowel transplantation (SBT).

We have previously demonstrated that functional innervation in vitro is a reliable indicator of subsequent viability of the small intestine following transplantation, and have therefore used this parameter to evaluate the effectiveness of different preservation solutions. Lactated Ringer's (LR) with no additive has shown encouraging results in rat bowel preservation.\(^11,12\) In search of a simple and effective preservation solution for small bowel we evaluated the cold preservation of rat ileum at 24 and 48 hours using: LR, Eurocollins (EC) and University of Wisconsin (UW). EC and UW were studied only at 48 hours since we have already reported the results of these
two solutions in preserving rat small bowel for 24 hours. All three solutions were consecutively studied without and with verapamil (LRV, ECV, UWV). Graft viability in each solution was studied by assessment of the physiological and pharmacological properties of smooth muscle and nerve.

MATERIALS AND METHODS

Male Lewis rats, weighing from 180 to 300 g, were fasted for one day prior to surgery, then anaesthetized with pentobarbital, 50mg/kg intraperitoneally. A total of 56 animals distributed in 9 groups with a minimum of 5 animals per group were used to study the different preservation times and solutions. A total colectomy was performed and the entire small bowel from the ligament of Treitz to the terminal ileum was harvested with a vascular pedicle consisting of the superior mesenteric artery on a cuff of proximal aorta, and of the superior mesenteric vein. The bowel lumen was flushed with 10 ml of the solution used for storage, which was previously chilled and supplemented with 0.5% neomycin sulfate. The intestinal vascular supply was then interrupted and the arterial lumen was immediately perfused with 5 ml of chilled preservation solution. Approximately 25 cm of the SB was divided into 2 segments, then stored for 24 and 48 hours at 4°C in LR and LRV, respectively. The same procedure, with only 48 h storage and the SB divided into 4 segments, was used for four other groups using the following solutions: EC, ECV, UW, UWV. At the end of the preservation period, 5 mm segments of the stored bowel were removed for physiological and pharmacological studies of the smooth muscle and nerve. A separate group (n=9) of control animals was sacrificed and the intestinal segments
were removed and studied immediately. A total number of 153 individual specimens of the intestine were evaluated during these experiments.16

Circular segments of ileum approximately 5 mm in width were mounted in isolated tissue baths and the contractile responses were recorded using Grass force transducers (model FT03C; Grass Instruments, Quincy, MA), and Beckman model RM dynographs (R511A, R611, Beckman Instruments, Inc, Fullerton, CA) using model 9853A couplers and filter settings of DC to 100 Hz. Each bath (20 ml) contained a Krebs physiological salt solution maintained at 37°C by a Haake heating pump (Haake Inc, Saddle Brook, NJ) that circulated warm water through an outer jacket surrounding each bath. The baths were equipped with parallel platinum electrodes connected to a Grass S48 stimulator for field stimulation, and the solution was bubbled with 95% O2 and 5% CO2. The composition of the Krebs solution in mmol/L was: NaCl 118.2, KCl 4.7, MgSO4 1.2, KH2PO4 1.2, NaHCO3 25.0, CaCl2 2.5, choline chloride 0.036, calcium disodium ethylene diamine tetraacetate 0.21, and dextrose 10.0. The parameters used to evaluate tissue viability were as follows: excitatory (Exc) and inhibitory (Inh) innervation in response to electrical field stimulation (20 Hz, 1 ms delivered from the Grass S48 stimulator)17, spontaneous myogenic activity (SMA) and muscle contractility (CR) using carbachol. Muscle sensitivity to the cholinergic agonist was calculated by determining the dose of carbachol that produced 50% of the maximal response (ED50), from a cumulative dose-response curve using a range of concentrations from 10^-7 to 10^-3M. The negative logarithm of this dose is referred to as the pD2 and larger numbers reflect a greater sensitivity. Statistics have been calculated using the one-way analysis of variance (ANOVA) to evaluate small bowel inhibitory activity for more than two solutions and the student’s t test for two solutions. A p < .05 was considered significant.
RESULTS

Control intestine exhibited SMA at an average rate of 30.6 ± 3.0 oscillations per minute. All samples were responsive to the cholinergic agonist carbachol, and to electrical field stimulation (figure 1). Exc predominated in all samples and Inh was also demonstrated in the majority of cases following the carbachol-induced increase in tone. All grafts preserved in solutions containing verapamil (figures 2 and 3) showed no SMA or Exc, a weakened CR but an intact Inh. At 24 h of storage in LR, the intestine retained an excellent degree of physiological function, but a loss of almost 50% of innervation was found with the longer preservation time of 48 hours. The addition of verapamil produced a significant increase in the inhibitory response after 48 hours of cold storage, at which time Inh was almost uniformly preserved by LRV (table 1, figure 4). For the same preservation period (48 h) there was no significant difference between results obtained with EC and UW (figure 5) and both solutions were somewhat better than LR alone. The inhibitory activity was better preserved in LRV than EC (p < .05) But there was no significance difference between LRV and UW.

When verapamil was used with EC and UW, SMA and Exc were blocked, CR was weaker and Inh was found intact as shown in figure 3 and table 2. After 48 h storage time in ECV, UWV, and LRV, no significant difference was found in the rat small bowel inhibitory activity between any of the 3 solutions. Muscle was less responsive to carbachol in all solutions containing the calcium channel blocker verapamil. Although the magnitude of contractions was smaller, and some samples were nonresponsive, the pD₂ of contracting samples was relatively unaltered, the greatest alteration occurred in UW solution (table 3).
DISCUSSION

LR is an effective medium to be used for 24 hr preservation of rat small bowel. This may be of considerable practical significance, if confirmed in human studies, as LR is universally available at minimal cost, in contrast to the more complex formulas tested. Kokudo et al\textsuperscript{11} demonstrated that LR alone was superior to UW and EC after 18 hour preservation of the rat small bowel used in syngeneic orthotopic transplantation. Verapamil, which has been used to improve liver preservation time and function,\textsuperscript{18,19} proved to be an excellent additive to protect the inhibitory activity which is most susceptible to ischemic damage incurred during storage,\textsuperscript{15,17,20} and LR was actually superior to the other complex solutions once verapamil was added. As expected, this calcium channel blocker decreased or eliminated those responses requiring muscle contraction, and we therefore had to rely on the status of inhibitory innervation to estimate the viability of the stored intestine. However, our previous studies\textsuperscript{13,14,21} conclude that this is a reliable indicator as it is, in fact, the most easily damaged component when the intestine is exposed to unfavorable conditions, and maintenance of inhibitory innervation during storage is uniformly associated with a high success rate upon subsequent transplantation. We are currently comparing LR to other simple solutions and attempting to improve LRV with the addition of cytoprotective agents and free radical scavengers, using the rat small bowel model of transplantation.
Polygraph tracings showing: 1) spontaneous myogenic activity (30/min) 2) electrical field stimulations and 3) dose dependent response to carbachol. Arrows indicate delivery of carbachol to the bath; numbers indicate logarithmic molar concentration. Calibration bars: vertical: tension, g., horizontal: time, 1 minute.
1 and 2: LR, 3: LRV. Polygraph tracings showing spontaneous myogenic activity, field stimulation (S) and dose dependent responses to carbachol. Arrows indicate delivery of carbachol to the bath; numbers indicate logarithmic molar concentration. Note the presence of Inh but blockade of SMA, Exc and CR in the tissue exposed to verapamil. Calibration bars: vertical: tension, g. horizontal: time, 1 minute.
Figure 3. **RAT ILEUM PRESERVED FOR 48 H: UW**

1. Rat nileum preserved for 48 h: UW

2. Polygraph tracings showing spontaneous myogenic activity (25/min), field stimulations (S) and dose dependent response to carbachol. Arrow indicates delivery of carbachol to the bath; numbers indicate logarithmic molar concentration. Note that only Inh and a weak cholinergic contraction are present in UWV. Calibration bars: vertical: tension, g., horizontal: time, 1 minute.
Table 1. **PHYSIOLOGICAL RESPONSES OF RAT ILEUM AT 24 AND 48 HOUR IN LR AND LRV**

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<td>N</td>
<td>5</td>
<td>6</td>
<td>5</td>
<td>10</td>
<td>9</td>
</tr>
</tbody>
</table>

B : Blocked with verapamil

% : Percent of samples responding

S : Number of samples examined

N : Number of animals studied

* With no previous storage
Table 2.

PHYSIOLOGICAL RESPONSES AT 48 HOUR WITH EC, UW, ALONE OR WITH VERAPAMIL

<table>
<thead>
<tr>
<th></th>
<th>EC</th>
<th>ECV</th>
<th>UW</th>
<th>UWV</th>
</tr>
</thead>
<tbody>
<tr>
<td>SMA</td>
<td>95</td>
<td>B</td>
<td>100</td>
<td>B</td>
</tr>
<tr>
<td>CR %</td>
<td>100</td>
<td>75</td>
<td>88</td>
<td>65</td>
</tr>
<tr>
<td>Exc %</td>
<td>74</td>
<td>B</td>
<td>78</td>
<td>B</td>
</tr>
<tr>
<td>Inh %</td>
<td>58</td>
<td>83</td>
<td>61</td>
<td>76</td>
</tr>
<tr>
<td>S</td>
<td>19</td>
<td>12</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td>N</td>
<td>7</td>
<td>6</td>
<td>5</td>
<td>8</td>
</tr>
</tbody>
</table>

B : Blocked with verapamil

% : Percent of samples responding

S : Number of samples examined

N : Number of animals studied
Table 3.

SMOOTH MUSCLE SENSITIVITY OF RAT SMALL BOWEL AFTER 48 HOURS STORAGE

<table>
<thead>
<tr>
<th>Preservation solutions</th>
<th>Sensitivity, pD₂</th>
<th>Preservation solutions</th>
<th>Sensitivity, pD₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.93 ± 0.3</td>
<td>___________</td>
<td>___________</td>
</tr>
<tr>
<td>LR</td>
<td>4.97 ± 0.2</td>
<td>LRV</td>
<td>4.74 ± 0.4</td>
</tr>
<tr>
<td>EC</td>
<td>4.83 ± 0.3</td>
<td>ECV</td>
<td>4.83 ± 0.6</td>
</tr>
<tr>
<td>UW</td>
<td>5.13 ± 0.3</td>
<td>UWV</td>
<td>4.18 ± 0.07</td>
</tr>
</tbody>
</table>

Sensitivity of rat intestinal smooth muscle to carbachol. Values are mean ± standard errors of pD₂ determinations in control intestine and after 48 hours preservation in different solutions.
Figure 4: Effect of verapamil on the inhibitory innervation following 48 hour preservation of rat small intestine in LR and LRV.
Figure 5: Physiological responses of rat ileum preserved for 48 h in LR, LRV, UW and EC. Note that in LRV, CR is weaker and Exc is blocked.
Figure 6: Inhibitory innervation of rat small bowel preserved in LRV, ECV, and UWV for 48 h.
REFERENCES


CHAPTER 3

EFFECTS OF PRESERVATION IN LACTATED RINGER'S, PHOSPHATE BUFFERED SUCROSE, EUROCOLLINS AND UNIVERSITY OF WISCONSIN SOLUTIONS ON THE FUNCTION OF RAT ILEUM AFTER SUBSEQUENT TRANSPLANTATION
EFFECTS OF PRESERVATION IN LACTATED RINGER'S, PHOSPHATE BUFFERED SUCROSE, EUROCOLLINS AND UNIVERSITY OF WISCONSIN SOLUTIONS ON THE FUNCTION OF RAT ILEUM AFTER SUBSEQUENT TRANSPLANTATION.

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Key words: Preservation of Rat Small Bowel, Survival, LRV, PBS, EC, UW.
Since there is presently no curative treatment for people with irreversibly inadequate small bowel (SB) function, SB transplantation would be beneficial in many intestinal disorders such as necrotizing enterocolitis, aganglionosis and other causes of short bowel syndrome. Although such transplantation is technically feasible, a specific method for intestinal preservation during the interval between procurement and implantation of the graft has not yet been developed; this currently restricts the procedure to situations where donor and recipient are in the same hospital.

In contrast to the other transplantable organs, the intestine contains an intrinsic nervous system, which must remain active following the transplantation if the intestine is to function. Neuronal damage during ischemia may be a sensitive predictor of less obvious alteration in other types of cells, which would produce a nonviable graft. We have therefore utilized the viability of intestinal neurons in vitro as an indicator of successful tissue preservation following cold storage, and have previously tested a series of solutions for their ability to preserve rat small intestine. Lactated Ringer's, a simple, inexpensive and readily available solution was reasonably effective, particularly with the addition of the calcium channel blocker verapamil, when compared to Eurocollins (EC) and University of wisconsin (UW) solutions, more complex and expensive alternatives currently used for preservation of other organs. To continue these studies on intestinal preservation, we compared these three solutions, supplemented with either verapamil or free radical scavengers (Superoxide dismutase and catalase), for the ability to preserve bowel function during 48 hours of cold storage, and in the current studies we utilized graft viability two weeks after transplantation as the indicator of successful preservation. For comparison, we also evaluated phosphate-buffered sucrose (PBS) without additives after 24 hour preservation.
MATERIALS AND METHODS

A total of 38 Lewis male rats received intestinal transplants in a syngeneic fashion to eliminate the effects of either rejection or GVHR. The donor and recipient surgery was performed as previously described. After the graft was harvested from the donor, the bowel lumen was flushed with 10 ml of chilled preservation solution containing 0.5% neomycin sulfate. With quick resection of the small intestine and its vascular pedicle, the lumen of the superior mesenteric artery was immediately perfused with 5 ml of the chilled storage solution. Solutions tested were LR, EC, and UW, supplemented either with verapamil "V" (25 µg/ml) or superoxide dismutase plus catalase "S + C" (5000 IU/5ml each given directly into the graft). The bowel was stored at 4°C in the corresponding solution for 48 hours, then transplanted into the recipient. The survivors were sacrificed 12 to 14 days after implantation. The graft and an identical segment of recipient bowel were extirpated for physiological and pharmacological studies of the smooth muscle and nerve in vitro. Graft status was also assessed by histological examination of the transplanted tissue and rate of recipient survival after transplantation. A control group (n=5) with transplantation performed immediately after extirpation of the graft was included in the studies.

For experimental analysis of graft function, circular rings of 5 mm in width were mounted in 20-ml tissue baths filled with a modified Krebs solution bubbled with 95% O₂ and 5% CO₂ which was kept at 37°C by a Haake heating pump (model FJ, Haake in., Saddlebrook, NJ) that circulated warm water through an outer jacket surrounding each bath. The composition of the Krebs solution (mmol/L) was: NaCl 118.2, KCl 4.7, MgSO₄ 1.2, KH₂PO₄ 1.2, NaHCO₃ 25, CaCl₂ 2.5, choline chloride 0.036, calcium EDTA 0.21, and dextrose 10.0.
One end of the specimen was fixed to the holder and the other end was connected to a Grass force transducer (model FT03C; Grass Instrument, Quincy, MA). Each bath contained parallel platinum electrodes connected to a Grass S48 stimulator for field stimulation. Trains of stimuli lasting for 30 seconds were delivered to each specimen every 4 minute. Responses were recorded on Beckman dynographs (model R511A and R611, Beckman instruments, Inc, Fullerton, CA) using model 9853A couplers and filter settings of DC to 100 Hertz.

The following parameters were evaluated: spontaneous myogenic activity (SMA), muscle contractility (CR) to the cholinergic agonist carbachol, excitatory (Exc) and inhibitory (Inh) responses of the smooth muscle to neurotransmitter released by electrical field stimulation (20 Hz,1 ms). Survival rates and physiological responses were expressed for each group as the percent of specimens that responded positively. Drug sensitivity to carbachol was quantified by determining the dose that produced 50% of maximal response, the negative logarithm of this dose being referred to as pD2, with larger numbers reflecting a greater sensitivity to the drug. Statistical significance was tested using the one-way analysis of variance (ANOVA). A $p < .05$ was considered significant.

RESULTS

Typical responses of the rat ileum to electrical field stimulation are illustrated in figure 1. There was essentially no difference between the control graft which was transplanted without prior storage and the graft which had been preserved for 2 days in LR with verapamil (V), indicating complete preservation of function during the cold storage. In contrast, excitatory innervation is lacking in the intestine stored for only 24 h in PBS. Macroscopically 30% of
the specimens extirpated from segments preserved in PBS, showed a purulent mucosal secretion and 40% showed fibrotic sections whereas grafts from all other groups exhibited no macroscopic changes from the control samples. Samples from preserved grafts showed no significant differences in oscillation frequency of SMA when compared to control, SMA was present in 100% of samples in all groups except for PBS in which 69% of samples showed SMA (table 1). Specimens from all groups contracted in a dose-dependent manner to the cholinergic agonist carbachol, except for those stored in PBS and UWV which had 69% and 75% activity, respectively. Specimens stored in EC had the greatest sensitivity to carbachol which was maximal with added verapamil (table 2). Functional innervation, particularly the nonadrenergic inhibitory innervation (Inh), was the most seriously affected physiological parameter examined in this study (table 1, figures 2-4). Both EC and UW with additives produced slightly better results than LRV but the differences were not statistically significant.

**DISCUSSION**

The alterations in intestinal function observed in grafts subjected to prior cold storage are the results of inadequate tissue storage, since no functional impairment was demonstrated after immediate transplantation. Some of the preservation solutions prevented much of the ischemic damage during storage. Even after 48 hour preservation of rat small bowel, LRV, which is a simple formula, was reasonably effective. All solutions were markedly superior to PBS, as only 24 hour storage in this medium caused considerable tissue damage, both functionally and morphologically. Although PBS is suitable for organ storage in clinical
renal transplantation, it is clearly not effective for small bowel preservation, and in these experiments it provided a useful demonstration of the consequences of inadequate tissue preservation, even for a relatively short period of time.

One of the meaningful results of this study is the further confirmation of our in vitro screening method for the estimation of adequate tissue preservation during cold storage. From previous studies we predicted that LR, UW and EC, with the addition of verapamil, would all provide moderate protection against ischemic damage for up to 48 hours of preservation. In the presence of the calcium channel blocker, one is forced to rely on inhibitory innervation as the sole physiological indicator of viability, since the contractile responses are temporarily attenuated or blocked. The present data, obtained after 2 weeks of transplantation, confirms that maintenance of inhibition is indeed an accurate indicator of tissue preservation.

The demonstration that LRV can provide reasonable protection against intestinal damage during 2 days of storage reinforces the concept that a simple solution could be developed to preserve the small bowel for human transplantation. Compared to the more complex EC and UW, this would have several advantages: greater availability, less cost and elimination of the problems associated with solutions high in K⁺. The latter must be flushed from the vasculature of the donor intestine prior to transplantation, to avoid detrimental effects on cardiac electrophysiology from the significant quantity of K⁺ entering the recipient circulation. This additional flush is potentially damaging to the vascular endothelium of the graft.
Additional studies on intestinal preservation are clearly required. Further modifications of both LR and the more complex solutions should be tested in the rat model and the most promising formulas should then be evaluated using human bowel in vitro.
**Table 1.**

**PHYSIOLOGIC RESPONSES OF TRANSPLANTED RAT SMALL INTESTINE.**

<table>
<thead>
<tr>
<th>Sols</th>
<th>Number</th>
<th>SMA</th>
<th>Exc</th>
<th>CR</th>
<th>Inh</th>
<th>Survival</th>
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<tbody>
<tr>
<td></td>
<td>A</td>
<td>S</td>
<td>%</td>
<td>%</td>
<td>%</td>
<td>%%animals</td>
</tr>
<tr>
<td>Ctl</td>
<td>5</td>
<td>15</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>87</td>
</tr>
<tr>
<td>PBS</td>
<td>10</td>
<td>13</td>
<td>69</td>
<td>46</td>
<td>69</td>
<td>15</td>
</tr>
<tr>
<td>LRV</td>
<td>5</td>
<td>10</td>
<td>100</td>
<td>80</td>
<td>100</td>
<td>60</td>
</tr>
<tr>
<td>UWSC</td>
<td>5</td>
<td>8</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>75</td>
</tr>
<tr>
<td>UW-V</td>
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<td>8</td>
<td>100</td>
<td>100</td>
<td>75</td>
<td>75</td>
</tr>
<tr>
<td>ECV</td>
<td>6</td>
<td>14</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>71</td>
</tr>
<tr>
<td>EC-SC</td>
<td>2</td>
<td>6</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
</tbody>
</table>

Ctl: Control

A: Number of animals studied

S: Number of samples examined

%: percent of responsive samples

Note: Samples were stored in all solutions for 48 h, except for PBS, where preservation time was 24 h. (EC-SC results are preliminary)
Table 2.

### CHOLINERGIC RESPONSIVENESS OF GRAFT

<table>
<thead>
<tr>
<th>Preservation solutions</th>
<th>pD₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4.93 ± 0.3</td>
</tr>
<tr>
<td>PBS</td>
<td>4.87 ± 0.3</td>
</tr>
<tr>
<td>LRV</td>
<td>4.23 ± 0.3</td>
</tr>
<tr>
<td>ECV</td>
<td>6.17 ± 0.4</td>
</tr>
<tr>
<td>UWV</td>
<td>4.80 ± 0.3</td>
</tr>
<tr>
<td>EC-S+C</td>
<td>5.37 ± 0.1</td>
</tr>
<tr>
<td>UW-S+C</td>
<td>4.91 ± 0.5</td>
</tr>
</tbody>
</table>

Sensitivity of rat intestinal smooth muscle to the cholinergic agonist carbachol in circular preparations preserved for 24 h in PBS and 48 h in the remaining solutions, then subsequently transplanted. Values are mean ± standard errors of pD₂ determinations.
A) Control, B) LRV-48, C) PBS-24. Each polygraph tracing illustrates spontaneous myogenic activity (oscillations/min), field stimulation (S) showing excitatory innervation, cumulative dose response with carbachol, and inhibitory innervation revealed with stimulation following the carbachol-induced increase in tone. Arrows indicate delivery of carbachol to the bath; numbers indicate logarithmic molar concentration. Calibration bars: vertical: tension, g. horizontal: time, 1 minute.
Figure 2: Physiologic responses of rat small bowel transplants Evaluation of PBS-24 and LRV-48.
Figure 3: Inhibitory innervation of rat ileum transplants: Comparison of 3 primary solutions with added verapamil. Except for control, all grafts were stored for 48 hours in the indicated solution before implantation.
Figure 4: Functional innervation of rat ileum transplants stored 48 hours in different solutions before implantation.
BIBLIOGRAPHY


CHAPTER 4

*In vitro* function of smooth muscle and nerve in human small bowel stored in Eurocollins, University of Wisconsin, and lactated Ringer's with verapamil
IN VITRO FUNCTION OF SMOOTH MUSCLE AND NERVE IN HUMAN SMALL BOWEL STORED IN EUROCOLLINS, UNIVERSITY OF WISCONSIN AND LACTATED RINGER WITH VERAPAMIL.

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Key words: Human Ileum Preservation, Graft Viability, Transplantation, EC, UW, LR+verapamil.
Small bowel transplantation (SBT) has slowly gained acceptance as a treatment for short gut syndrome (SGS), especially when complications of long-term total parenteral nutrition (TPN) occur. TPN, the only treatment for patients with SGS, limits the life-style of the patients and their families, and depends on maintaining long-term venous access which can be a major problem because of catheter-related thrombosis and infections, especially in children. Although a transplanted segment of small intestine would be extrinsically denervated, the intrinsic nervous system responsible for normal function would be largely intact if an adequate preservation was carried out. Autonomic reinnervation of graft is also possible after SBT.

At the present there is no simple and effective method for preservation of the small intestine, while other organs such as kidney can be stored for several days, permitting transport to an appropriate recipient after removal. To try and formulate an effective cold storage solution for adequate preservation of the small bowel, we have previously developed a screening technique which relies on the presence of intact innervation in vitro to indicate tissue viability following storage. Using the rat syngeneic model of intestinal transplantation, we found that functional innervation was an excellent predictor of subsequent transplanted graft survivability and function. Having tested different solutions, we concluded that Lactated Ringer's was a promising candidate for subsequent modification and that free radical scavengers and calcium channel blockers were both beneficial additives for an intestinal preservation solution. In the present experiments we use the same viability test on human ileum and evaluate the effectiveness of Eurocollins (EC), University of Wisconsin (UW) and lactated Ringer's + verapamil (LRV) as preservation solutions.
MATERIALS AND METHODS

Immediately after harvesting other organs for transplantation, segments of human small bowel were obtained from each of 16 organ donors, through the Montreal transplantation team at different hospitals where donors were available. Initially, the abdominal vasculature was flushed with chilled, oxygenated UW solution and the tissue was transported on ice to the laboratory. Once at the laboratory, the fresh harvested bowel was studied as control (1 to 5 h). The remaining ileum was divided into 3 segments, for storage in each of the 3 different preservation solutions: UW, EC and LRV. The lumen of each segment was flushed with its chilled preservation solution and the segment was stored in a container with the corresponding oxygenated solution at 4°C. The concentration of verapamil was 25 µg/ml. Between 6 and 15 hours later, 15 mm tissue strips (5 mm in width) were cut from circular rings removed at multiple locations of the stored small bowel. The containers with the remaining bowel segments were reoxygenated and returned to storage. The strips were then mounted in 20-ml isolated tissue baths containing a modified Krebs solution, which was bubbled with 95% O₂ and 5% CO₂, maintained at 37°C for physiological and pharmacological studies. The temperature of the Krebs solution in the baths was maintained by a Haake heating pump (model FJ, Haake inc., Saddlebrook, NJ) and its composition was: NaCl 118.2, KCl 4.7, MgSO₄ 1.2, NaHCO₃ 25.0, CaCl₂ 2.5, choline chloride 0.036, dextrose 10.0 and calcium EDTA 0.21 (all components are from Fisher Scientific Company, Canada, and values are in mmol/L). One end of each specimen was connected to a Grass force transducer (model FT03); Grass Instruments, Quincy, MA) with silk, and the other end was fixed to the holder. The tissue was mounted with an initial tension of 1.0 g. Each
bath contained parallel platinum electrodes connected to a Grass S48 stimulator for field stimulation. Trains of stimuli lasting for 30 seconds were delivered every 4 minutes to each tissue. Tension was continually recorded using Beckman models RM, R511A and R611 ink-writing dynographs (Beckman instruments, Inc, Fullerton, CA) using model 9853A couplers and filter settings of DC to 100 Hz. After approximately 60 minutes, the background tone and the response of the tissue to intermittent transmural electrical field stimulation (TEFS) stabilized, and the experiment was begun. Tissue responses to TEFS and to cumulative doses of the cholinergic agonist carbachol (10^-7 to 10^-3 mol/L) were determined. The following compounds were also studied: phentolamine, propranolol, and atropine (in mol/L; 10^-4, 10^-5, 10^-5, respectively). The effectiveness of each preservation solution was assessed using the following parameters of tissue viability: spontaneous myogenic activity (SMA), muscle contractility (CR) to the cholinergic agonist carbachol (10^-7 to 10^-3 M), excitatory (Exc) and inhibitory (Inh) responses to electrical field stimulation (20 Hz, 1 ms). Sensitivity to carbachol was measured by administration of multiple doses and determining the dose that produced 50% of maximal response. The negative logarithm of this dose is referred to as pD\_2 and larger numbers reflect a greater sensitivity to carbachol.

Subsequent tests were similarly executed approximately every 24 hours on additional segments until specimens would show no more activity. A total of 214 tissue samples were examined and specimens from responsive groups were studied for up to 105 hours. Statistical significance was tested using the one-way analysis of variance (ANOVA) to evaluate inhibitory activity of the preserved small bowel.
RESULTS

Analysis of the data on SMA, Exc, CR, and pharmacological responsiveness of intestinal smooth muscle indicated no significant differences between the specimens in the control group and those preserved in EC and UW for different periods of time (figure 1 and 2). The human ileum contracted in a rhythmic manner, and the spontaneous myogenic activity showed a frequency of 6.1 ± 0.4 oscillations per minute (figure 1). When the tissue was exposed to electrical field stimulation, both excitatory and inhibitory innervation could be demonstrated by eliciting contractile and relaxant responses; the latter predominated during the elevation in tissue tone following administration of carbachol.

Experimental results following storage were grouped into 6 different periods of time, 1-5h, 6-15h, 20-39h, 43-63h, 66-80h, and 86-105h. All samples preserved in UW and EC, regardless of the preservation time maintained the SMA, CR and Exc as previously mentioned, but these parameters could not be evaluated for tissue stored in LRV, since calcium channel blocker, as expected, interfered with contractile responses. This alteration is reversible; after transplantation of rat small intestine stored in LRV, contractile function was restored in bowel transplants studied after 2 weeks of recipient survival. Inhibitory innervation therefore becomes the crucial variable in estimating tissue viability after storage in solutions containing verapamil. In both control and preserved bowel, the inhibitory response was not altered by either the α-blocker phenolamine (10^4 mol/L each) or the β-blocker propranolol (10^5 mol/L), which indicates the absence of an adrenergic component. In all specimens, regardless of the preservation time and solution, atropine 10^5 partly blocked the Exc and produced a marked relaxation (decrease in tone). Tissue sensitivity to carbachol
remained quite stable during preservation in UW for all periods of time. In contrast, sensitivity decreased with time in EC, and tissue stored in LRV showed an expected inconsistency. Functional innervation (particularly Inh) was the most affected physiological parameter following storage in all 3 solutions (figure 3-5). LRV maintained moderately good Inh at 20-39 hour, and many samples remained active for up to 43-63h. Of the three solutions tested, EC maintained the best Inh response after preservation for short periods (6-15h), but the differences were not statistically significant. UW was the solution in which the bowel retained Inh for the longest preservation period, and maintained significantly greater Inh at 43-63h than did the other 2 solutions (p < .05).

DISCUSSION

In order to be successful, intestinal transplantation requires a preservation medium which can maintain functional innervation of the graft in optimum condition for a period long enough to allow time for recipient preparation and HLA typing. In this study, none of the 3 solutions demonstrated such capacity. Currently there is not yet an ideal solution for human small intestinal preservation before transplantation.

From these experiments LRV, EC and UW seem to give similar results for brief storage of human small bowel and the length of adequate preservation period using these solutions was shorter in human than in the rat. We can not determine the reason for this discrepancy, but it may be significant that tissue obtained from human was not harvested in optimum conditions as it was for the rat specimens. The rat, indeed, represents a good model in which to study bowel preservation for transplantation; parameters of graft viability and
responses to biologically active compounds showed similar patterns of response in both rat and human small bowel. LRV, EC and UW appear to be suitable media to be further improved in the attempt to develop an adequate method for small bowel preservation before transplantation.
**Table 1.**

**INHIBITORY INNERVATION OF HUMAN ILEUM PRESERVED IN UW, EC, LRV.**

<table>
<thead>
<tr>
<th>Solutions</th>
<th>Time (Hour)</th>
<th>N (Samples)</th>
<th>Cases</th>
<th>Inhibition % samples</th>
</tr>
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<tbody>
<tr>
<td>CONTROL</td>
<td>1-5</td>
<td>11</td>
<td>5</td>
<td>91</td>
</tr>
<tr>
<td>UW</td>
<td>6-15</td>
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<td>EC</td>
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<td>88</td>
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</tr>
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<td>LRV</td>
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<td>5</td>
<td>13</td>
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<td>UW</td>
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<td>19</td>
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<tr>
<td>EC</td>
<td>86-105</td>
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<td>3</td>
<td>0</td>
</tr>
<tr>
<td>LRV</td>
<td>86-105</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

Samples stored in UW and EC retained SMA, Exc and CR in all groups.
CHOLINERGIC RESPONSIVENESS OF PRESERVED HUMAN ILEUM.

<table>
<thead>
<tr>
<th>Time hour</th>
<th>6-15</th>
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<th>43-63</th>
<th>66-80</th>
<th>86-105</th>
</tr>
</thead>
<tbody>
<tr>
<td>UW</td>
<td>5.23 ± 0.3</td>
<td>5.15 ± 0.3</td>
<td>5.58 ± 0.3</td>
<td>5.62 ± 0.3</td>
<td>5.16 ± 0.3</td>
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<td>10</td>
<td>10</td>
<td>10</td>
</tr>
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<td>EC</td>
<td>5.30 ± 0.3</td>
<td>5.02 ± 0.2</td>
<td>5.59 ± 0.5</td>
<td>5.70 ± 0.6</td>
<td>6.33 ± 0.2</td>
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<td>N</td>
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<td>5</td>
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<td>LRV</td>
<td>5.37 ± 0.3</td>
<td>6.50 ± 0.4</td>
<td>4.69 ± 0.5</td>
<td>5.30 ± 0.7</td>
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Table 2. Sensitivity of human intestinal smooth muscle to carbachol in circular preparations preserved for different periods of time. Values are means ± standard errors of pD2 values.

Note: control identical to UW 6-15h.
Figure 1: Control (1-5 h storage).

Example of polygraph tracings showing: 1) spontaneous myogenic activity (6/min) 2) electrical field stimulations and 3) dose dependent response to carbachol. Arrows indicate delivery of carbachol to the bath; numbers indicate logarithmic molar concentration. Calibration bars: vertical: tension, g., horizontal: time, 1 minute.
Figure 2.

A) 43-63h UW, B) 66-80h C) 86-105h. Each polygraph trace showing: spontaneous myogenic activity, field stimulations (S) and dose dependent response to carbachol. Arrows indicate delivery of carbachol to the bath; numbers indicate logarithmic molar concentration. Calibration bars: vertical: tension, g., horizontal: time, 1 minute.
Figure 3: Inhibitory response of human small bowel stored in LRV
Figure 4: Inhibitory response of human small bowel preserved in EC solution.
Figure 5: Inhibitory innervation in Human small bowel stored in uw
BIBLIOGRAPHY.


CHAPTER 5

DISCUSSION AND CONCLUSION.
The present work indicates that the rat is an acceptable model in which to study preservation solutions for human small bowel transplantation. Physiological and pharmacological properties of rat and human small bowel can be evaluated in vitro as good indicators of an effective subsequent transplantation. In search of a suitable SBP solution, we have been able to demonstrate the effectiveness of various solutions on the rat SB for 48 hours. This study also demonstrates the usefulness of cytoprotective agents in cold storage, as seen with the very simple LRV.

Previous studies in our laboratory with EC and UW have shown poor animal survivability and impairment of the inhibitory response, after transplantation of all the grafts previously preserved for 48 h. Animal survival was less than 40% using both solutions, and only UW preserved some excitatory neuron activity (33%). In this study, when CCB or FRS were added to both solutions (EC and UW) during preservation for 48 h before transplantation, improvement in animal survival and Inh was remarkable (tab 1, chap 3).

The calcium channel blockers have been shown to prevent hepatic cell injury caused by cytosolic calcium overload, and inclusion of calcium channel blockers in the preservation fluid was shown to improve the functional results of kidney preservation. Therefore, we examined the effects of the calcium channel antagonist verapamil on function of smooth muscle and nerve of rat and human SB after cold storage.

Free radical scavengers were used because reperfusion injury is thought to be mediated, at least in part, by oxygen free radicals, which play a role in the acute phase of multiple organ failure. Sun et al reported an enhanced SBP using SOD in conjunction with UW solution. They found a significant improvement in the recovery of absorptive function, morphology,
and transplant survival of rat small intestine after preservation of 18 hours, which suggested the importance of amelioration of free radical-induced injury in maintaining the integrity of preserved SB. Dalsing et al. have also reported protection by SOD from a form of necrotizing enterocolitis in weanling rat pups. They found that untreated rats developed transmural necrosis of the intestine within 48 hours following only 1 minute of superior mesenteric artery occlusion, while SOD treatment provided substantial protection. This demonstrated the role of superoxide radicals in the initial mucosal lesion and the evidence of a potential for SOD as a clinically useful agent for the prevention of transmural bowel necrosis.

The biological activity of nitric oxide, which may also scavenge superoxide, is enhanced by FRS (SOD), therefore in this study, we assume that S + C might have acted to improve NO activity, and minimize reperfusion injury by decreasing the production and increasing the elimination of oxygen free radicals.

Procurement of rat and human small bowel was done in different conditions; the human bowel underwent a lot of manipulation before being harvested, while rat tissue suffered minimum stress. This alone may not explain the discrepancy in tissue survivability between the two models, and caution is necessary in direct extrapolation from the rat either about the efficacy or lack thereof of any agent or method as it may apply to the human small bowel. Southards and collaborators have pointed out that the rat may not be a suitable animal for research on free radical scavengers. They found that the ratio of superoxide dismutase to xanthine oxidase in the rat liver was much lower than in the dog or human and suggested that the rat liver could be more sensitive to oxygen-derived free radical damage than in other
species. Sonnino et al\textsuperscript{93} maintained that the rat model is only partly comparable to the clinical short gut syndrome. Intestinal resections that are lethal in humans permit rats to survive after a period of weight loss. We think that results obtained from the rat, a model resistant to sepsis, could be too easily obtained and should not be overestimated with regard to what might happen in humans.

Notwithstanding these reservations, the present research design appears to be an excellent one for use in the evaluation of preservation solutions for SBT. Graft viability response patterns were similar in both rat and human, only the length of preservation was different (better in rat).

We have been able to demonstrate that preservation solutions for the small bowel need not be complex to be effective. LR, a very simple solution, with addition of verapamil showed better results than those obtained previously with the two complex formulas, EC and UW.\textsuperscript{49} In agreement these findings, Kokudo et al\textsuperscript{73} showed better preservation in LR than in UW and EC after 12 h, as demonstrated by animal survival, and by enterocyte and crypt cell function.

When compared to \( S + C \), verapamil, which is a much less expensive agent, produced similar preservation of the inhibitory innervation, and a better rat survival than did previous graft storage for 48 h in UW. Secondary effects of verapamil in preservation solutions (temporary blockade of SMA, Exc, and decrease of CR) did not interfere with normal rat intestinal function after transplantation. None of the effective solutions (EC and UW with additives, LRV) used in rat small bowel preservation for 48 h before transplantation are recommended for human SBP, as they did not reliably protect the innervation for more than
15 hours. It is suggested that preservation for at least 24 h is needed for human bowel, because typing in SBT may be necessary for identical matching as in bone marrow transplantation. It is probable that verapamil combined with S + C might have a beneficial synergistic effect in SBP. We therefore suggest additional experiments on SBP, using simple solutions like LR or PBS with additives, to be conducted on human tissue since there have been almost no experimental studies on human small intestinal preservation in the literature.
BIBLIOGRAPHY


