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TWELVE-HOUR AND TWENTY FOUR–HOUR PRESERVATION OF SMALL BOWEL ALLOGRAFTS BY SIMPLE HYPOTHERMIA

SURVIVAL UTILIZING CYCLOSPORINE

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Canine small bowel was harvested and stored by simple hypothermic technique. After 12- and 24-hr storage, respectively, the small bowel graft was allotransplanted into recipients. All animals receiving 12-hr stored grafts (n=9) survived beyond 5 days. In the 24-hr storage group, 67% of the animals (n=9) survived beyond 5 days. Successful storage for such extended periods by simple hypothermia has not been achieved previously. Donor pretreatment with antibiotics as well as extensive intraluminal irrigation of the harvested small bowel are considered to be important technical features in this successful preservation.

Successful clinical allotransplantation of the small bowel awaits solutions to numerous problems, including graft rejection, graft-versus-host reaction, suboptimal graft function, and storage (*1*). It has not been possible previously to store small bowel allografts by the preferred simple hypothermic technique beyond a few hr. Small bowel allotransplantation, and more particularly its storage, is affected by several features peculiar to this solid organ: its extreme sensitivity to ischemia, somewhat ameliorated by its great regenerative capacity; its exposure to the outer environment, including microorganisms, on a chronic basis; and the constant contact with pancreatic and other proteolytic enzymes that bathe it, providing a source of ready and rapid tissue injury during ischemic conditions. Numerous publications have appeared pertaining to the "enteric death" that occurs in patients as well as experimental animals from massive bowel necrosis as a complication of shock and hypoperfusion.

Lillehei (*2*) was unable to extend ischemic storage of small bowel beyond 5 hr utilizing simple hypothermia. Longer storage required complicated techniques (*3–7*), often combined with perfusion, pharmacologic supports, or hyperbaric oxygenation. We report herein successful 12-hr and satisfactory 24-hr preservation of small bowel allotransplants utilizing simple hypothermic storage at 2°C. Cyclosporine was used for immunosuppression.

MATERIALS AND METHODS

Healthy mongrel dogs (13–20 kg body weight) were used as donors and recipients. The animals were pretreated with oral neomycin (2 g/day for 4 days) and kanamycin (0.5 g IM, the night before transplantation) and were fasted for 24 hr preoperatively. Kanamycin (1 g) and cephamandol (1 g) were given parenterally on the morning of surgery. Small bowel allotransplants were exchanged between 2 dogs where feasible.

Donor surgery. Under general anesthesia (sodium pentobarbital), laparotomy was done by midline incision. The entire small bowel was isolated on a vascular pedicle consisting of superior mesenteric artery and vein. After systemic heparinization (200 U/kg), the intestine was flushed in situ through the superior mesenteric artery with 200 to 300 ml lactated Ringer's solution at ambient temperature, resected, cooled by intravascular flush solution at 4°C, and irrigated intraluminally with cold (4°C) lactated Ringer's solution. Intravascular flush solution contained heparin (5000 U/L) and intraluminal irrigant contained kanamycin (0.5 g/500 ml). Volumes of 500–700 ml of intravascular flush solution and 500–1000 ml of intraluminal irrigation solution were used. The intestine was rinsed before storage and immersed in a small amount of the same fluid in a plastic container surrounded by ice slush. The container was stored in a commercial refrigerator at 4°C until the time of transplantation. The temperature inside the container proved to be 0–2°C.

Recipient surgery. The stored intestine was flushed again briefly with a small amount (20–50 ml) of heparinized cold lactated Ringer's solution. The underperfused portions were resected (i.e., approximately 15 cm at each end) before allotransplantation. The graft was implanted orthotopically. The continuity of digestive tract was restored by 2 end-to-end anastomoses. A 15 cm-length Thiry-Vella loop of proximal jejunum was isolated, and the ends were exteriorized for monitoring purposes. After irrigation of the abdominal cavity with a copious amount of warm fluid to combat hypothermia, the incision was closed in layers.

Parenteral fluid was given to the dogs for 4 or 5 days, and antibiotics (including penicillin, kanamycin, and oral neomycin) were given for 7–10 days postoperatively. Intravenous cyclosporine was given to allograft recipients from the first postoperative day, starting with a dosage of 16 mg/kg/day and tapering to 8 mg/kg/day by the eighth postoperative day, in 2 equally divided doses. After 1 month, oral cyclosporine (40 mg/kg/day) was substituted. Prednisone (5–10 mg/day) was added to the regimen after 2 weeks.

Eighteen dogs received stored allotransplants. Duration of cold storage was 12 hr in 9 allografts and 24 hr in an equal number.

Total protein and albumin in the serum, hemoglobin, and hematocrit were determined at 10, 20, and 30 days after surgery and monthly thereafter. At 1 month, a D-xylose absorption test was performed in survivors (n=7). D-Xylose (0.5 g/kg in 300 ml of water) was given orally, and blood levels were determined by the spectrophotometric method of Roe and Rice (*8*). Samples were taken at 0, 20, 40, 60, 90, and 120 min after administration.

In 2 animals, cyclosporine absorption curves were determined following an oral dose of 15, 25, or 35 mg/kg. Blood samples in EDTA were taken at 0, 1, 2, 3, 4, 6, and 12 hr, and *whole* blood levels were measured by radioimmunoassay (Sandoz). In 6 animals receiving daily doses of oral cyclosporine, blood samples were taken approximately 15 hr after the evening dose, and trough *serum* levels were measured by radioimmunoassay. Samples were refrigerated until the time of separation and measurement.

All laboratory data were compared with those of autotransplants (n=6), and allotransplants (n=5) with a nonstored graft transplanted at the same period as the experimental group.

Numerical values in the text represent the mean ±SD.

RESULTS

The 18 recipients of the experimental group and the 11 animals in the control groups tolerated surgery, recovered from anesthesia, and lived at least 2 days. All animals (100%) with a 12-hr stored allograft survived more than 5 days; the longest survival was 107 days (n=1). Six animals (67%) with a 24-hr stored bowel survived more than 5 days, including 1 70-day survivor (Fig. 1). The mean survival was 45.4±38.9 days in the 12-hr storage group and 22.9±27.1 days in the 24-hr storage group ($P<0.10$). There were no early (<5 days) deaths in the 12-hr group but 3 in the 24-hr group. The cause of these 3 early (≤5 days) deaths was found to be graft necrosis. Later (>6 days) deaths in both groups were attributed to renal failure (n=3), rejection (n=5), arterial thrombosis (n=1), graft necrosis (n=1), or systemic infection (n=2). In 3 animals, the cause of death could not be determined.

Survivors with stored bowel living beyond 8 weeks (n=5, including 3 animals from the 12-hr storage group and 2 from the 24-hr storage group) were compared to allografted controls with nonstored bowel (n=5, 86–140-day survivors), and with autotransplants (n=6). Initially, body weight decreased similarly in all groups. Body weight in the nonstorage group stabilized by 3–4 weeks while the storage group continued to lose weight beyond this time (Fig. 2).

Serum total protein of the storage group was slightly lower than that of the nonstorage group or autotransplants (Table 1). Hemoglobin and hematocrit were similar in all groups.

D-xylose absorption test at 1 month showed a similar pattern among storage, nonstorage, and autotransplanted animals. Peak xylose blood levels (34.1±7.3 mg/dl) of the storage group (n=7) were slightly lower than those of the nonstorage group (39.1±10.7 mg/dl) (n=5) or those of autotransplants (40.7± 11.8 mg/dl) (n=6). But all were significantly lower than the peak levels of normal animals (72.8±12.0 mg/dl) (n=11) (Fig. 3).

Seven animals were treated with oral cyclosporine from 22–44 days after surgery. Trough serum levels were 357±302 (range, 87–1090) ng/ml from 12 determinations in 6 animals surviving 28 to 93 days following allotransplantation. Cyclosporine absorption curves were determined in 2 animals. They showed similar curves to those of nonstored allografts. However, sometimes the peak time was delayed or the peak increment was smaller and, therefore, the curves appeared less predictable.

DISCUSSION

Because of the great regenerative capacity of the intestinal mucosa, the small bowel can withstand longer warm ischemia than other solid organs, such as the liver or the heart. Warm ischemia of up to 7 hr with subsequent recovery of an isolated segment of small bowel has been successfully demonstrated under carefully controlled conditions (*9*). However, ischemia beyond 4 hr may result in chronic damage, with the development of fibrosis or stenosis of the segment of small bowel in the postischemic period (*10*). Despite the relatively long warm ischemic time that is possible with small bowel, simply hypothermic storage of the small bowel beyond the safe warm ischemia time (approximately 4 hr) has not been possible. Lillehei (*2*) was able to store small bowel by simple hypothermia for only 5 hr. Cold storage could be successfully extended up to 48 hr with additional adjuvants such as chlorpromazine or hyperbaric oxygen (*6, 7*), however. Utilizing perfusion storage rather than simple hypothermia, Toledo-Pereyra (*4*) was able to store small bowel beyond 24 hr. Our results in preserving small bowel for up to 24 hr by simple hypothermia, without the use of adjuvants, metabolic inhibitors, or cumbersome perfusion techniques, may be attributable to pretreatment of donor intestine, the in situ flush procedure, and immediate intraluminal irrigation before storage. These aspects of the technique are believed to reduce both perfusion endotoxemia and other infectious complications, as well as enzymatic damage to the unperfused bowel during storage. It has been convincingly shown that endotoxemia is the basis of irreversible shock that occurs in experimental animals following prolonged intestinal ischemia (*11*). Nonabsorbable antibiotics administered prophylactically before the onset of small bowel ischemia have been reported to prevent endotoxic shock (*12*). Similar experimental findings have been elucidated and confirmed by other workers utilizing a variety of experimental settings. Toledo-Pereyra (*13*) has suggested that antibiotic usage reduced the incidence of hemorrhagic necrosis following storage. The role of pancreatic enzymes in exacerbating ischemic injury of the small bowel cannot be overlooked. So-called "trypsin enteritis" is a manifestation of advanced mucosal injury from the digestive action of intraluminal trypsin, chymotrypsin, and elastase on ischemic small bowel. Bounous (*14*) has shown that intraluminal elastase was largely responsible for the loss of brush border proteins in ischemic enteropathy. Intraluminal irrigation prior to the onset of experimental ischemia successfully reduced the loss of brush border proteins (*14*). Better preservation of enterocytes with intraluminal irrigation has also been demonstrated previously (*15, 16*).

The mechanism of ischemic injury of the small bowel and, indeed, of many other solid organs is inadequately understood. The role of superoxide radicals in ischemic small bowel injury has been elucidated recently by Granger (*17*) and Grøgaard

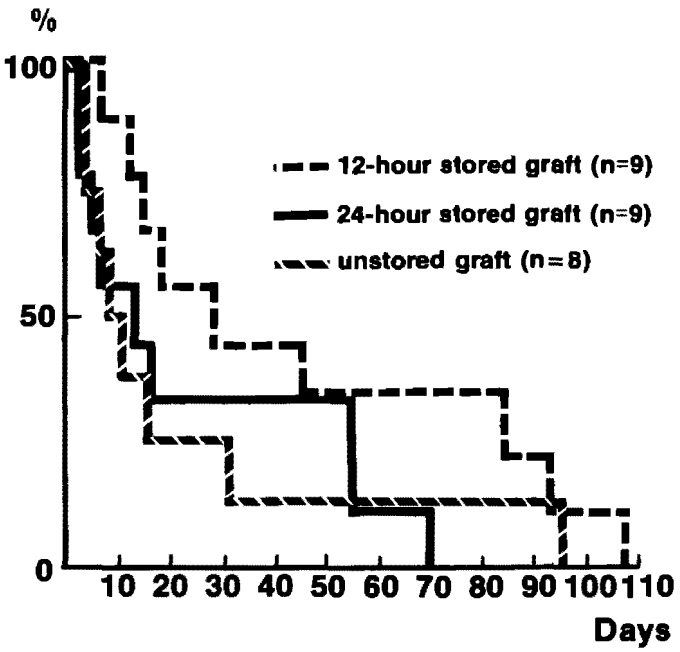

FIGURE 1. Survival of animals receiving stored and unstored grafts.

(*18*). Increased capillary permeability is thought to be a major mechanism of such injury (*17*). Increased permeation of macromolecules could occur as a result of increased capillary permeability (*18*). Lysosomes, endotoxin, and prostaglandins are believed to play an adjuvant, but not a primary, role in inducing capillary injury during ischemia. On reperfusion, hypoxanthine accumulated during the ischemic period may react with xanthine oxidase to produce superoxide radicals. Thus, ischemic injury may progress further during reperfusion (*18*).

The functional capacity of stored small bowel following transplantation was inferior to that of unstored fresh allografts. It is highly probable that storage-related mucosal damage seen in stored small bowel (unpublished data) is responsible for this difference in functional capacity. The extent of mucosal denudation seen in some transplanted specimens is certainly striking. We are currently investigating the possibility that mucosal healing and functional restoration could be hastened by temporary isolation of the transplant loop from pancreatic secretions and food passage.

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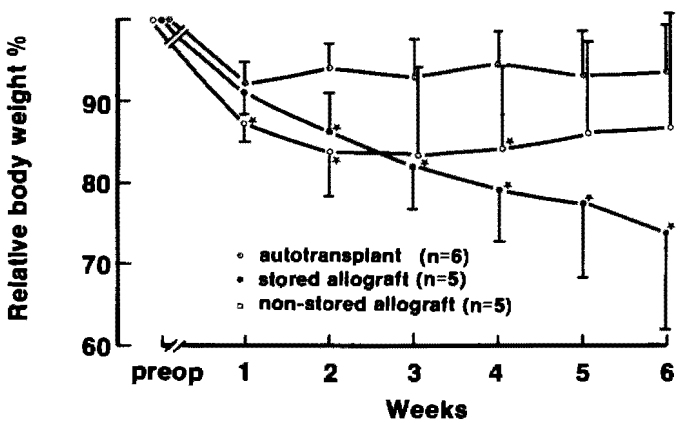

FIGURE 2. Body weight change following entire small bowel transplantation among longer (> 8 wks) survivors of autotransplants (n=6), stored allografts (n=5), and nonstored allografts (n=5). Animals with a stored graft continued to lose weight. The bars represent SD. Star (★) indicates $P<0.05$ vs. autotransplants.

TABLE 1. Total protein, serum albumin, hemoglobin, and hematocrit among longer (beyond 8 weeks) survivals[a]

	Protein	Albumin	Hemoglobin	Hematocrit
Autotransplant with nonstored graft, n=6/6	5.7±0.7	2.4±0.2	11.8±1.1	38.2±3.0
	5.8±0.8	2.4±0.1	12.2±1.8	40.3±5.7
Allotransplant with nonstored graft, n=5/5	6.3±0.5	2.7±0.6	10.5±2.0	33.8±6.8
	5.9±0.6	2.6±0.4	11.7±1.1	37.2±3.8
Allotransplant with stored graft, n=5/3	5.2±1.0	2.4±0.7	11.0±1.5	36.5±5.3
	5.1±0.7	2.2±0.4	10.1±1.4	34.3±4.6

[a] Values are expressed as mean ± SD. Value above the solid line indicates 1-month results and the value below, 2-month results.

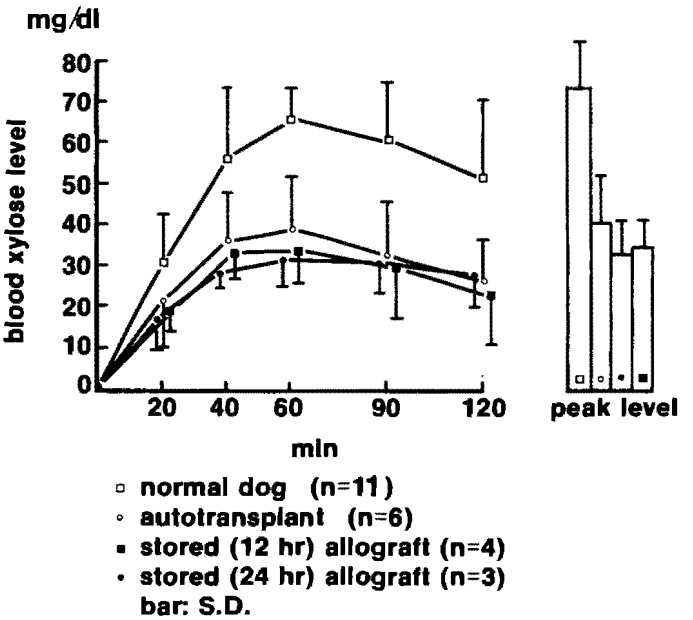

FIGURE 3. D-Xylose absorption test at 1 month following transplantation. Normal nontransplant animals (n=11) are compared with autotransplants (n=6), 12-hr stored allografts (n=4), and 24-hr stored allografts (n=3). All transplant groups had similar absorption levels that were significantly lower than normal controls.

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