CASE REPORT

Preservation injury of the small bowel graft in clinical small bowel transplantation

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Abstract

Clinical success of small bowel transplantation depends on quality of the preservation small bowel graft which is notoriously sensitive to ischemia. There is still no general agreement as to which segment of the small bowel is preferred (jejunum or ileum) for clinical use. In our study, using a light microscopy and concentrations of tissue serotonin-positive cells, we tried to identify a part of the human intestine, which is more resistant to preservation injury sustained by HTK preservation solution with 1–24 hr of cold ischemia. Statistical analysis of both parameters did not reveal any significant differences between the jejunum and ileum. According to our data, there is no difference between jejunal and ileal grafts in susceptibility to ischemic injury due to cold ischemia within 24 hours when using HTK preservation solution. A significant difference was observed in histological pictures only after 12-hour of cold ischemia in both groups (jejunum and ileum) *(Fig. 2, Ref. 11)*. Full Text (Free, PDF) *www.bmj.sk*.

Key words: small bowel transplantation, preservation injury, serotonin.

Clinical success of small bowel transplantation depends on quality of the preservation small bowel graft which is notoriously sensitive to ischemia. Small bowel transplantation (SBT) is intended primarily for patients with the short bowel syndrome in cases where long-term parenteral nutrition is not the optimal therapeutic option (1). Although recently improvements have been made, rejection, infection, preservation, and reperfusion injury continue to be a barrier to the successful SBT (2). Early postoperative complications, including endotoxemia, bacterial translocations, and stimulation of the recipient's immune response have been attributed to preservation injury (3). Knowing the segmental small bowel transplantation, living-related transplantation has been initiated. The small bowel consists of jejunum and ileum; both parts are notoriously sensitive to ischemia. However, there is no agreement which segment of the bowel should be preferred (4). Our study was aimed at preservation of jejunum and ileum after a perfusion in the multiorgan procurement. We tried to identify a part of the intestine, which is more resistant to preservation injury. The means for evaluation of the preservation injury included light microscopy and concentration of tissue serotonin-positive cells in small bowel mucosa.

Material and methods

Intestinal specimens were acquired from six multiorgan donors after a standard preparation, perfusion with histidine-tryptophan-ketoglutarate (HTK) solution and explantation (5). The mean donors age was 58 years (range 32–71 years). Three donors died due to intracranial bleeding, one died due to head injury, and two died due to asphyxia.

Studied groups

The obtained intestines were divided into jejunum and ileum. Five specimens, 10-cm long, were cut from each part of the bowel.

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These specimens were stored in HTK solutions for 1, 6, 9, 12, and 24 hours. After this period, biopsy samples were obtained from the jejunum and ileum, and morphological injury was evaluated using histological examination and concentration of serotonin-positive cells.

Histological examination – light microscopy

Samples were fixed in a 10 % buffered formalin solution, embedded in paraffin, cut 4 μm thick, and stained with hematoxylin-eosin. Histological damage was assessed using Park's histological classification for a damaged bowel (6). Histological staining was evaluated by 2 independent observers who were blinded to the source of the specimens.

Immunohistochemistry – 5HT-positive cells

Immunohistochemistry was performed on the 4 μm-thick paraffin sections of biopsies. Slides were deparaffinized in xylene and re-hydrated in graded ethanol. After the deparaffinization and rehydratation, slides were cooked in a microwave oven using 0.01 M citrate buffer pH 6.0 for target retrieval. Endogenous peroxidase was blocked by 0.3 % H_2O_2 in 70 % methanol for 30 minutes. The tissues were then incubated with a 10 % horse serum (Vector laboratories, Burlingame, CA) for 20 min to prevent unspecific binding and FcR binding. A primary antibody (anti Serotonin, DakoCytomation, Denmark), diluted 50x, was applied for 30 minutes. On negative control slides, the step with monoclonal antibody was omitted. Detection of monoclonal antibody was done using biotinylated horse anti mouse IgG (H+L) (Vector laboratories, Burlingame, CA) diluted 200x for 30 min. Then specimens were incubated with R.T.U. Vectastain Elite ABC Reagent (Vector laboratories, Burlingame, CA) for 30 min. Finally, specimens were stained with 3,3 diaminobenzidine (Serva, Germany) for 5 min and were counterstained with Harris's hematoxyllin before they were embedded in Entellan (both from Merck, Germany).Immunohistochemical staining for serotonin was evaluated by two independent observers who were blinded to the source of the specimens. Serotonin-positive cells were counted in 20 high-power fields (400x). Results are expressed as positive cells per 10 HPF (high-power fields).

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Results

Concentration of serotonin-positive cells

After the evaluation of serotonin-positive cells in jenunum, the average number of positive cells was 80.0 (SD=10.1) after the 1-hour preservation, 68.5 (SD=14.1) at 6 hours, 65.0 (SD=7.69) at 9 hours, 56.5 (SD=8.16) at 12 hours, and 66.6 (SD=12.16) at 24 hours. There was not a significant difference between individual preservation periods (ANOVA repeated measures and grouping factor). In the ileal specimens, the serotonin-positive cell count was 62.0 (SD=11.2) at 1 hour, 57.3 (SD=14.4) at 6 hours, 56.3 (SD=15.8) at 9 hours, 48.5 (SD=14.8) at 12 hours, and 48.8 (SD=16.3) at 24 hours. Again, no significant difference was observed between individual preservation periods (ANOVA repeated measures and grouping factor). Statistical analysis of preservation periods in jejunum and ileum did not demonstrate a significant difference with the $p<0.05$ (ANOVA repeated measures and grouping factor) (Fig. 1).

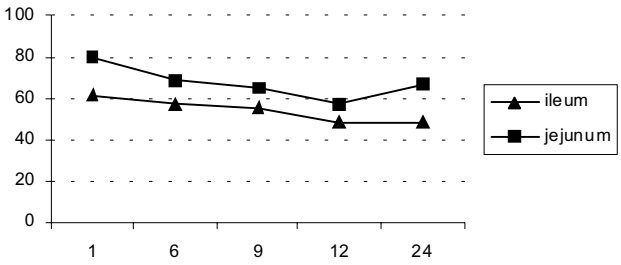

Fig. 1. Concentration of serotonin-positive cells in jejunal and ileal grafts.

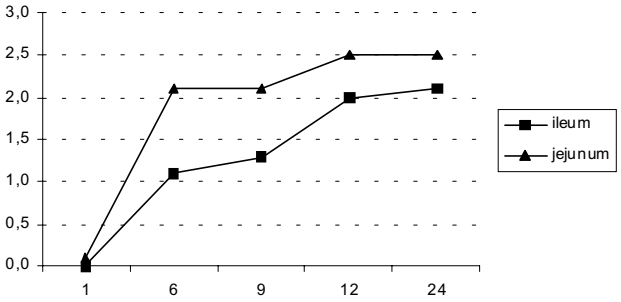

Fig. 2. Grade of morphological injury in jejunal and ileal grafts.

Evaluation of the histological damage

In the evaluation of the histological damage of jejunum, the mean degree of injury was 0.2 (SD=0.41) after a 1-hour cold ischemia, 2.2 (SD=0.41) at 6 hours, 2.2 (SD=0.41) at 9 hours, and 2.5 (SD=0.55) at 12 and 24 hours. A significant difference was noted between 1 hour and 12 hours and between 1 hour and 24 hours of cold ischemia with the $p<0.05$ (Friedmans test). In the evaluation of the histological damage of ileum, the mean degree of injury was 0.0 (SD=0.0) after 1-hour cold ischemia, 1.2 (SD=0.75) at 6 hours, 1.3 (SD=0.82) at 9 hours, 2.0 (SD=0.63) at 12 hours, and 2.2 (SD=0.41) at 24 hours. Again, a significant difference was noted between 1 hour and 12 hours and between 1 hour and 24 hours of cold ischemia with the $p<0.05$ (Friedmans test). In neither group a statistical analysis of individual cold-ischemia times showed a significant difference (Mann-Whitney test) (Fig. 2).

Discussion

The small bowel is very sensitive to ischemic injury and optimal graft preservation is imperative, since early postoperative complication might be caused by insufficient organ preservation.

Although the main changes in the small bowel occur after reperfusion, it depends on the duration and condition of the preservation period.

Clinical small bowel transplantation is currently performed using two methods, cadaveric and living related transplantation. Cadaveric transplantation usually involves the entire small bowel. Most centers recommended the use of living donor ileum (10, 11). Jejunal grafts have been also used but the procedure is technically challenging. However, there is still no agreement which segment of the bowel (jejunum, ileum) should be used for living related transplantation (4).

In our study, we focused on the human small bowel preserved in HTK solution 1–24 hour after the multiorgan procurement. The aim of the study was to determine, which part of the human small bowel (jejunum vs. ileum) is more resistant to prolonged ischemic periods. Two parameters were used, histology as a gold standard for the evaluation of mucosal damage in the small bowel graft, and evaluation of serotonin-positive cells.

Regarding histology, the standard scheme developed by Park (6) was used; this grading system was successfully used for evaluating animal and human small bowel injury (9). Use of serotonin for predicting the success of transplantation is based on the fact that injury of the bowel wall is associated with injury to enterochromaffin cells releasing 5-HT from their destroyed granules causing decreased serotonin levels in the bowel (9). Serotonin (5-hydroxytriptamine, 5-HT) is a biogenous amine which is up to 95 % present in the enterochromaffin cells of the gastrointestinal tract (8).

Takeyoshi et al assessed mucosal damage and recovery of the small intestine after the transplantation using overall status, survival rate, electrophysiology, biochemistry and morphology. In summary, an extensive damage of the mucosa in a canine model after the 24-hr cold preservation with Ringer lactated solution and transplantation normalizes functionally within 3–7 days in the jejunum and 7–14 days in the ileum, whereas morphological recovery takes 28 days in the jejunum and more than 28 days in the ileum (7). Chang et al showed in the pig experimental small bowel transplantation, using UW solution and cold ischemic periods ranging from 0 to 12 hours, that the ileum is more resistant to preservation injury than the jejunum (based on light microscopy) (4). Up till now, no experimental study was published on ischemic injury of the human small bowel.

In this study, statistical evaluation of both parameters did not reveal any significant differences between the jejunum and ileum. Based on data obtained in our study, there is no difference between jejunal and ileal grafts in susceptibility to ischemic injury due to cold ischemia within 24 hours when using HTK preservation solution. A significant difference was observed in histological examination only after 12 hours of cold ischemia in both groups (jejunum and ileum). This data demonstrated that regarding living related transplantation, there is not a preferable part of small bowel, but technically most comfortable and recommended is ileum part of the small bowel. In cadaveric small bowel transplantation, bowel graft up to 12 hours of cold ischemia is optimal.

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