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The Effect of Varying Colloid Osmotic Pressure in the Luminal Preservation Solution During the Static Cold Preservation of Rat Intestines

Oltean M[1], Seradilla J[3], Padma A[2], Oltean M[1], Mackay Söfteland J[1]
[1]*Sahlgrenska University Hospital,* [2]*University of Gothenburg,* [3]*Hospital Universitario La Paz*

Background: The hallmark of intestinal preservation injury is progressive subepithelial edema, which may ultimately result in barrier disruption. The epithelial barrier is a semipermeable membrane and it is possible to use a number of suitable colloids to limit the development of subepithelial edema. Polyethylene glycols (PEG) are colloids that have the ability to bind loosely to biological membranes and to sequester large amounts of water. We wanted to increase the osmotic pressure to examine the effect of increasing luminal osmolality with a colloid while keeping the electrolyte content low.
Methods: Intestines of Sprague Dawley rats were perfused intravascularly with IGL-1 and procured. The lumen was flushed with solutions with a sodium concentration of 25 mEq and varying concentrations of PEG 3350Da (13,5%, 17%, and 20%). The osmolality of these solutions was 297, 490 and 563 mOsm/kg, respectively. Control intestines received only the vascular perfusion with IGL-1. Samples were taken after 8 and 14 hours of cold ischemia time (CIT). We compared the development of intestinal preservation injury in rats using the Chiu-Park score and Goblet cell (GC) counts. Tight junction proteins zonula occludens (ZO)-1 and claudin 3 were studied using immunofluorescence.
Results: Vascular perfusion and preservation with IGL-1 resulted in very advanced mucosal injury with median Chiu-Park score 7 (range 5-8) already after 8h of CIT. The injury further progressed to complete mucosal dissolution after 14 hours. In contrast, a low and stable injury score (median score 2-3) was found during the preservation period in all groups receiving luminal PEG 3350 solutions with no clear advantage seen with varying concentrations of PEG. At the molecular level, luminal PEG maintained the expression of both tight junction proteins studied throughout the fourteen hours of CIT. The different PEG concentrations with increasing osmolality did not appear to have an impact on the results. Beyond injury grading, other comparisons between the intestines receiving luminal preservation and control intestines (vascular perfusion with IGL-1 alone) were not possible due to the advanced tissue injury in the control group.
Conclusion: Luminal colloids provide a high level of mucosal protection and stabilization against the development of intestinal preservation injury. However, increasing the concentration of the colloid up to 20% with an osmolality of 563 mOsm/kg does not prevent subepithelial edema entirely.

P-06

Intra-tissue Immune Response in Pediatric Patients with Short Bowel Syndrome

Negri E[1,2], Sacchetti C[2], Zulli A[1,2], Cianci M[1,2], Beaubrun G[1,2], Ugolini S[1,2], Dell'Otto F[1,2], Coletta R[1], Morabito A[1,2]
[1]*Meyer Children's Hospital,* [2]*University of Florence*

Introduction: Short bowel syndrome (SBS) is a rare disease characterized by inadequate nutrient absorption caused by severe reduction of the intestinal mucosal surface.
The study immunologically characterized the bowel layers' lymphocyte T infiltrate and evidenced its alterations through a qualitative analysis.
Methods: Pediatric SBS patients who underwent bowel surgery between July 2018 and March 2019 were enrolled. Full thickness small bowel samples were taken and processed. The tissue was dissected and the tissue-infiltrating T lymphocytes (TILs) were isolated, cloned and expanded. The phenotypic characterization was performed using flow cytometry and anti-CD4 and CD8. The clones were stimulated and phenotypically characterized through ELISA test, evaluating the production of IFN-γ, IL-4, IL-17, IL-10.
Student's t-test was used for statistical analysis and statistical significance was considered as $p<0,05$.
Results: Twelve patients were enrolled, with a median age of 3 years and 9 months (range: 2 months - 18 years and 5 months) and a median length of the small intestine of 40 cm (range: 19 cm - 67 cm). All patients were parenteral nutrition dependent, 92% received also enteral nutrition and 58% received intestinal decontamination. The intestinal samples of ten of these patients were analyzed and TILs were isolated in 60% of cases. 49 T cell clones were obtained and the phenotypic characterization was made: 30 T helper lymphocytes (61.2%), 17 T cytotoxic (34.7%), 1 T double-positive $CD4^+CD8^+$ (2%), 1 T $CD4^-CD8^-$ defined γδ (2%). We proceeded with the functional characterization: T helper lymphocytes are divided intoTh1 clones (20, 80.0%), T reg (1, 3.3%), Tnull (5, 16.7%), no Th2 clone, no Th0, no Th17. Cytotoxic T lymphocytes are classified into: Tc1 clones (12, 70.6%); Tcnull (5, 29.4%); no Tcreg clone, no Tc2, no Tc0, no Tc17. The correlation between the number of isolated T cell clones and the lack of intestinal decontamination therapy was not statistically significant (p-value=0,27).
Conclusion: We hypothesize that the alteration of the immune balance of SBS patients, especially the acquisition of pro-inflammatory characteristics, is caused by the dependence on parenteral nutrition and the deprivation of enteral nutrition or by the intestinal dysbiosis.