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DOCTORAL THESIS

THE IMPLICATION OF BACTERIAL OVERGROWTH AND BACTERIAL TRANSLOCATION IN SHORT BOWEL SYNDROME.

ABSTRACT

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bacterial overgrowth, bacterial translocation, short bowel bacterial overgrowth, short bowel syndrome, extended bowel resection, ileocecal valve, intestinal failure, total parenteral nutrition, parenteral nutrition, parenteral nutrition associated liver disease, intensive care unit, enteral nutrition.

INTRODUCTION

The term „short bowel" was defined by Rickham in 1967 as a small intestinal remnant of 75 cm in a newborn, which equals 30% of normal small bowel length in that age group. In premature babies, this corresponds to 30% of the total calculated intestinal length for a given gestational age. Short bowel syndrome (SBS) is a state of significant maldigestion and malabsorption due to extensive loss of functional absorptive intestinal surface area. In born malformation such as gastroschisis, volvulus, atresia as well as necrotizing enterocolitis and intra-abdominal tumors are often subject to extended surgery leading to the resection of large bowel length. Before the introduction of parenteral nutrition in the 1960s most of these patients died from severe malnutrition and dehydration. Total parenteral nutrition (TPN) changed the outlook of SBS patients dramatically by providing nutritional support during the process of intestinal adaptation. Nowadays, the undisputed mainstay of therapy in short bowel patients is TPN through a central venous catheter, excellent survival has been demonstrated a in the short term. The main clinical challenge we face in SBS is in the management of many nutritional problems taking place as a result of malabsorption secondary to the reduced adaptative surface area. Following extended small intestinal loss, the symptoms of an individual patient depends on the absorptive capacities and the in born characteristics of the remaining bowel,

Many of the septic episodes in SBS were caused by enteric organisms suggesting that bacterial translocation from the remaining bowel play a pivotal role in the genesis of this infection. The histopathology findings in PNALD are progressive

intracellular and canalicular cholestasis, portal and lobular inflammation, macrophage hyperplasia, bile duct proliferation and fibrosis. Animal studies indicate that after massive intestinal resection, intestinal bacteria and their by-products translocated to the liver via the portal venous and lymphatic systems. Bacteria by-products inhibit hepatocellular bile acids transport and activated hepatic macrophages via locally produced cytokines. Once the PNALD becomes irreversible the only option left is combined liver and small bowel transplantation. More evidence now shows that the translocating bacteria from patient`s bowel cause significant part of these infections. BT occurred in MLNs of all the rats studied with extended bowel resection but the lesser the intestinal resection the more limited the BT to distant organs (liver and spleen). We did not observe systemic spread of bacteria, though this can happen as described in medical literatures when there is additional immunosuppression with depleted kupffer cells in the liver. The ultimate immune effector cells in the defense against bacterial translocation are the phagocytic cells (macrophages). Selected bowel decontamination with neomycin failed to abolish bacterial overgrowth and subsequent BT in our study. Animal experimental studies show that due to resection, the bowel loses its barrier function bacteria are then easily translocated from the intestinal lumen to the MLNs, liver and spleen thereby increasing the risk of systemic infection, though this might not be the case of clinical small bowel transplantation. Some species are more or less resistant against the antibiotics chosen, antibiotic treatments pave the way for their overgrowth. Some studies show that antibiotics treatment actually promoted bacterial translocation and increased the possibilities of yeast and other fungi growth in the intestine. Another alternative is treating bacteria with probiotics. A number of experiments indicate decreased bacterial translocation using probiotics. Medications that decrease intestinal transit time and support a normal secretion of saliva, gastric acid, pepsin, bile and pancreatic juice can also decrease the numbers of intra-luminal bacteria. The mucus layer of the intestine prevents pathogenic bacteria from adhering to enterocytes, which is the first step in BT. It also protects the intestinal mucosa from acids and other noxious agents and functions as an anchoring site for secretory immunoglobulin. Different strategies to directly boost the immune system have been adopted. These include vaccination and the subsequent production of specific antibodies, non specific activation of macrophages, treatment with immune cell nutrients (for example glutamine) and priming of the immune system with intestinal microflora (for example probiotics). Furthermore, the addition of secretory immunoglobulin A (IgA) may prevent bacterial translocation, but the relative contribution of this has not been clarified. More evidence now suggests that the host immune system is capable of limiting BT beyond the mesenteric lymph nodes. Three major factors promoting BT in the gut are the increased number of bacteria in the gut, the impaired immune function and the increased permeability of mucosal barrier. If all intestinal defense systems fail and bacteria or other microorganisms succeed in traversing the bowel wall other back-up systems quickly go to work, these are primarily the MLNs that scan the draining lymph. MLNs drain lymph and take part in the elimination of antigens. Some bacteria have

adapted strategies to escape recognition by the immune system. To what extent other bacteria can use similar approaches to survive is not entirely known, when one or few of these backup mechanisms fail to slow bacteria perhaps due to immunosuppression or too great a microbial load, then systemic infection can ensue. While bacterial translocation and its complications was proved in animal models its existence and importance in humans is difficult to ascertain. However, it is becoming generally accepted that BT is an important early step in the pathogenesis of opportunistic infections caused by endogenous intestinal flora

STUDY DESIGN

We simulated SBS in rats to in order to help us understand better what exactly happens in human SBS patients. Using rats was cost efficient and readily available.

Animals were allocated according to experimental groups (group A-D). 22 Rats were studies, 16 were operated (Group A. B & C), while 6 (Group D) had no surgery

Group A = 4 rats with 60% intestinal resection without ileocecal valve resection, and 4 rats with 60% intestinal resection with ileocecal valve resection.

Group B = 3 rats with 70% intestinal resection without ileocecal valve resection, and 3 rats with 70% intestinal resection with ileocecal valve resection.

Group C = 2 rats with 75% intestinal resection with ileocecal valve resection: 2 rats.

Group D: D1 = 2 non surgical rats with daily histamine 2 blockers/proton pump inhibitors, D2 = 2 non surgical rats with antibiotics/probiotics treatment, D3 = 2 non surgical control study rats.

OBJECTIVE

1. To determine in a group of experimental laboratory animals (rats) the possible predictors of SBBO confirmed by quantitative duodenal and stool cultures and to study the relationship between SBBO and bacterial translocation

2. To determine if therapeutic and anatomical diferences such as the presence of an ileocecal valve, treatment with gastric acid blocking medicines and gut decontamination medications were significantly associated with SBBO and subsequent BT.

3. To find out the relationship between liver function (as assesseed by hepatic transaminase level and bilirubin) the nutritional status (Serum albumin) with SBBO and subsequent BT. The relationship between SBBO can and intestinal villous atrophy and mucosal damage. Whether excess bacteria in the intestinal lumen

causes deconjugation of bile salt acids and if unconjugated bile acids in the duodenum can be corelated with the degree of SBBO.

4. To establish if the use of empiric antibiotics for bowel decontamination decreases episodes of sepsis.

5. To determine if gastric hypoclorhydria with prevalent use of proton pump inhibitors and histamine 2 blockers contributes to the development of excess bacteria burden.

6. To establish a significant relationship between positive duodenal aspirates and cecal stool cultures and subsequent risk of bacteria translocation to distant organs..

7. To examine the evidence that after extended small bowel resection adaptation takes place in animal models just like in humans and focused our attention on the factors that influenced this adaptation and the strategies used to optimize this process.

RESULTS

Bacterial overgrowth was found in duodenal fluid of greater than 100,000 colony forming per unit per ml. Duodenal fluid culture of rats prior to surgery were all negative for bacteria. Pathology samples of the rat intestines, MLNs, liver and spleen obtained at initial resection for comparison at the end of the experiment were normal.

Rats with 60% intestinal resection without ileocecal valve resection: Duodenal aspirate and cecal stool cultures were positive for Enterococcus 30 days postoperatively.

Histopathology result showed an increase in villous height and crypt dept, also the intestinal diameter thickness (mucosal hyperplasia and bowel dilatation), pseudo-stratified immature enterocytes and rare calciform cells (Figure 1).

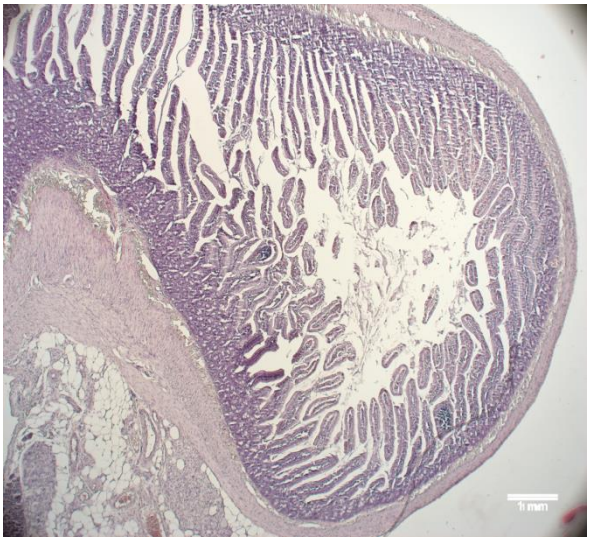


Figure 1: Villous height and crypt dept increased, and intestinal diameter thickness (mucosal hyperplasia and bowel dilatation). Rare calciform cells with pseudo-stratified immature enterocytes

Rats with 60% intestinal resection with ileocecal valve resection: Duodenal aspirate cultures were positive for Enterococcus, while cecal stool cultures were positive for Enterococcus and Klebsiella 30 days postoperatively. Histopathology result showed an increase in villous height and crypt dept, also the intestinal length thickness (mucosal hyperplasia and bowel dilatation), pseudo-stratified immature enterocytes with rare calciform cells and lymphoplasmocytic mucosal infiltration (Figure 2).

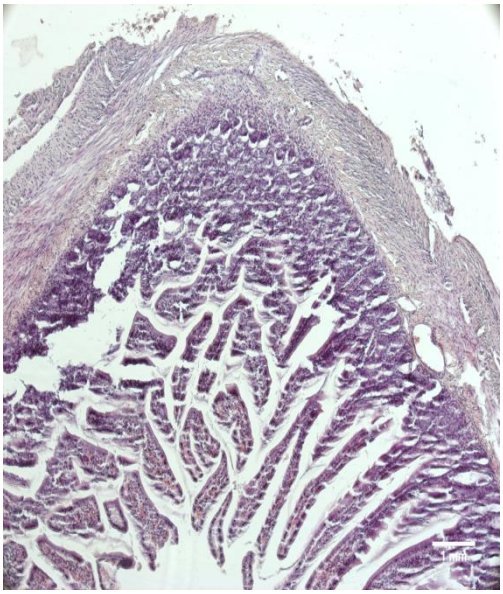


Figure 2: Villous height and crypt dept increased and intestinal length thickness (mucosal hyperplasia and bowel dilatation). Rare calciform cells with pseudo-stratified immature enterocytes,

Rats with 70% intestinal resection without ileocecal valve resection: Duodenal aspirate and cecal stool cultures were positive for Enterococcus 21 days postoperatively. Histopathology result showed villous atrophy and no calciform and detrusor cells (Figure 3).

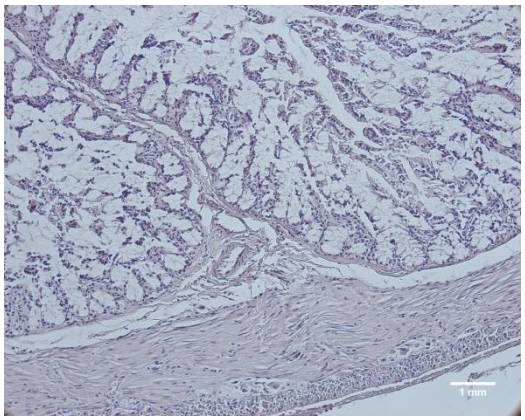


Figure 3: Villous atrophy and the absence of calciform and detrusor cells

Rats with 70% intestinal resection with ileocecal valve resection: Duodenal aspirate cultures were positive for Enterococcus, while cecal stool cultures were positive for Enterococcus and E. coli 21 days postoperatively. Histopathology result showed that villous atrophy increased with some calciform cells at the base of the intestinal villi (Figure 4).

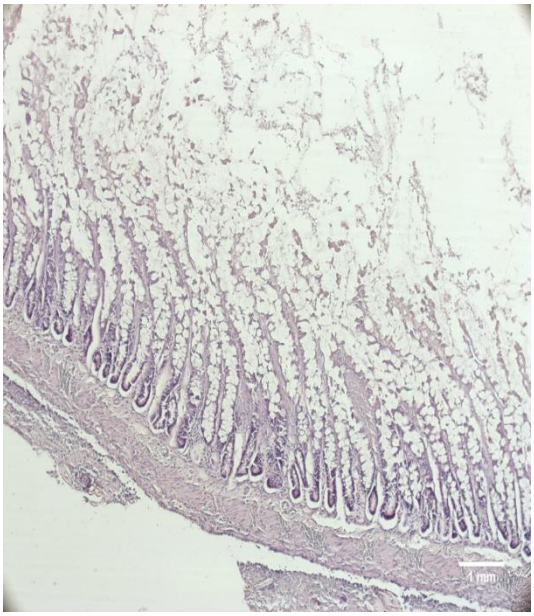
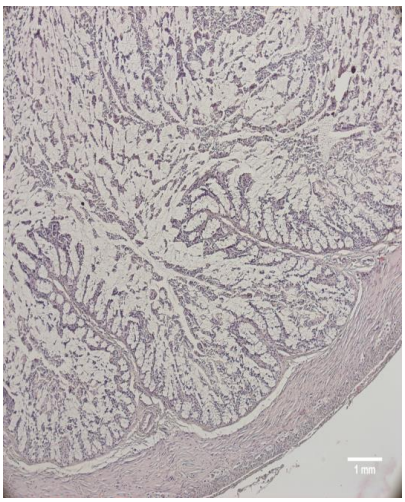


Figure 4: Villous atrophy increased with some calciform cells at the base of the intestinal villi.

Rats with 75% intestinal resection with ileocecal valve resection: Duodenal aspirate cultures were positive for Enterococcus, while cecal stool cultures were positive for Enterococcus and E coli 14 days postoperatively. Histopathology result showed that intestinal atrophy and proliferation of programmed gcells death (apoptosis) within enterocytes increased, mucosal hyperplasia with immature enterocytes and no calciform cells. Epithelial desquamation mixed with mucosal hyperplasia and leucocytes infiltration, intestinal glands hypertrophy with lumenal detrusor cells associated with rounded hypertrophied calciform cells, moderate and numerous areas of leucocytes infiltration. Leucocytes infiltration was associated with numerous detrusor cells at the lamina propria (Figures 5a and 5b).


A

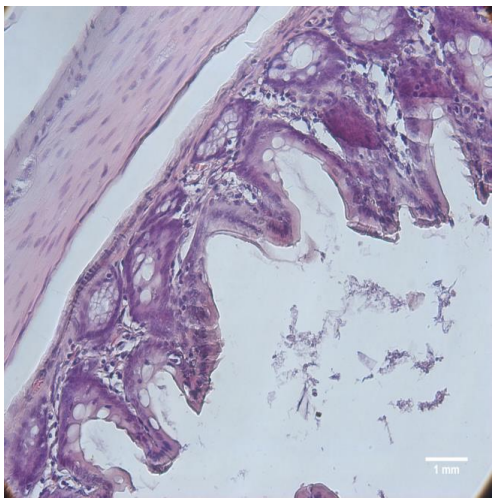

B

Figure 5: Increased intestinal atrophy and proliferation of programmed cells death within enterocytes, mucosal hyperplasia with immature enterocytes and absence of calciform cells, epithelial desquamation with mucosal hyperplasia, leucocyte infiltration and intestinal glands hypertrophy with lumenal detrusor cells associated with rounded hypertrophied calciform cells. Leucocyte infiltration associated with numerous detrusor cells at lamina propria

Non surgical rats with daily histamine 2 blockers/proton pump inhibitors and daily antibiotics/probiotics medication: Duodenal aspirate cultures were positive for Enterococcus, but cecal stool cultures were negative for Enterococcus, E. coli and Salmonella. Histopathology result of rats treated with histamine 2 blockers/proton pump inhibitors showed lymphoplasmocytic mucosal inflammatory infiltration, reduced number of calciform cells and numerous enterocytes proliferation (Figure 6).

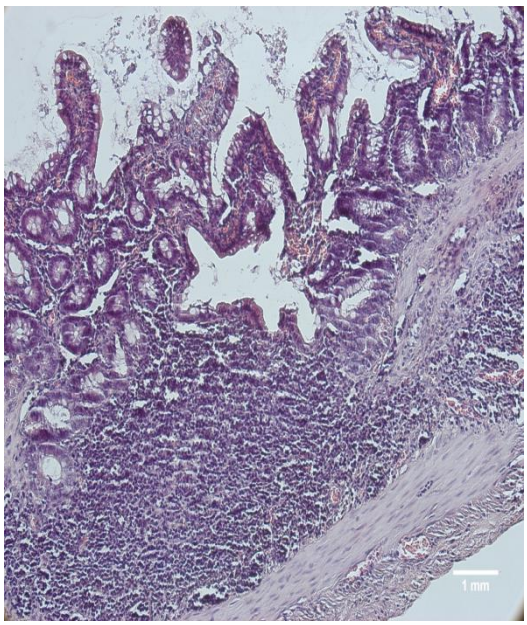


Figure 6: Massive lymphoplasmocytic mucosal inflammatory infiltration, reduced number of calciform cells, and numerous enterocyte proliferation, mild villous atrophy, hypotrophy, glandular dilatation and increased enterocyte atrophy

Histopathology result of rats that received probiotics/antibiotics showed a mild mucosal inflammatory infiltration and villous hypertrophy glandula dilatation and enterocyte atrophy (Figure 7).

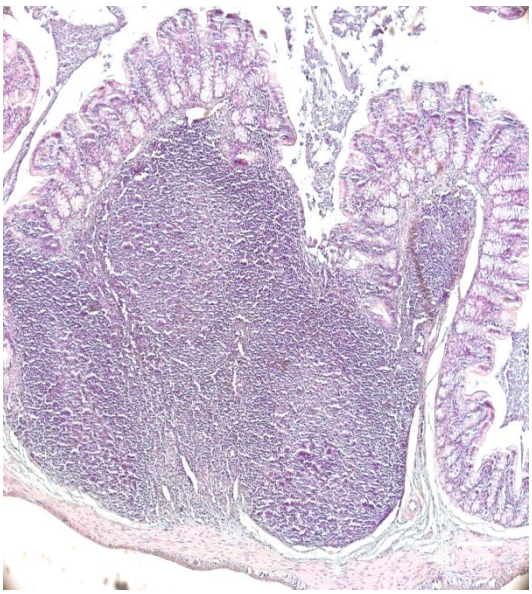


Figure 7: Mild mucosal inflammatory infiltration and villous hypertrophy

Control study rats: Duodenal aspirate cultures were negative for bacteria, but histopathology result showed intestinal villi without atrophy and no enterocyte hypertrophy (Figure 8).

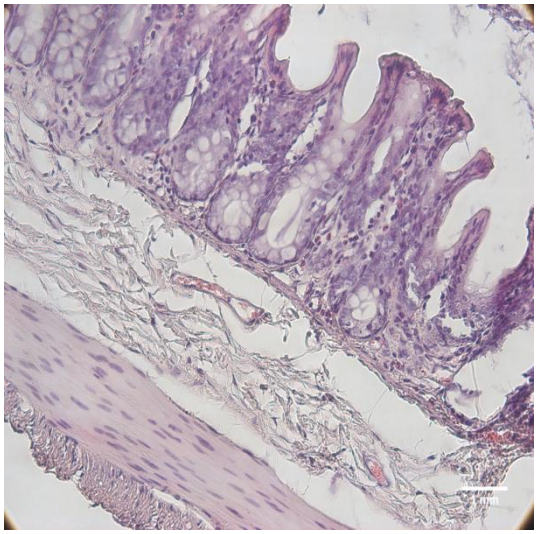


Figure 8: Intestinal villi without atrophy and no enterocyte hypertrophy

Microbiology culture (duodenal fluid, cecal stool, MLNs, spleen and liver), histopathology and laboratory tests clearly showed a milder form of SBBO and BT in rats with lesser intestinal resection compared to rats with extended intestinal resection. The lesser the residual bowel the lesser the chances of host survival. Bacterial concentration was higher in rats with ileocecal valve resection. The presence or the absence of the ileocecal valve helped determine subject adaptation. The percentage of the intestinal resection determined the degree of the BO and subsequently BT. The reason for this was not further studied but this pattern is consistent compared to other experiments. The lesser the intestinal resection the lesser the chances of BT to distant organs. Rats that received just probiotics/antibiotics treatment had milder form of SBBO and no BT compared to rats that received just proton pump inhibitors/antihistamine 2 blockers because the chronic inhibition of gastric acid secretion by histamine 2 receptor blockers/proton pump inhibitors increased the number of gastric bacteria. In case of extended intestinal resection in human extreme care should be taken to preserve as much healthy bowel as possible beacuse every little gut length conserved is essential for survival. The correlation between the percentage of the duodenal aspirate enterococcus and cecal stool bacteria in rats was R= 0.81. However, the correlation between duodenal aspirate enterococcus and cecal stool bacteria of rats without ileocecal valve resection compared to rats with ileocecal valve resection was R= 0.57. All these being statistically significant (Correlations were analyzed with Pearson`s correlation test)

CONCLUSION

1. We demonstrated how the residual intestine length after extended bowel resection in rats influenced the post-operation evolution of short bowel syndrome subjects.
2. We proved that extended bowel resection in rat`s triggered chain of reactions that led to the bacterial overgrowth, the abnormal intestinal mucosal morphologic, functional changes and bacterial translocation to distant organs.
3. We did not find systemic spread of bacteria in rats after extended bowel resection or endotoxins translocated from the bowel (with abundant gram negative bacteria) to the blood without showing positive blood culture.
4. After extended bowel resection BT to the MLNs occurred in almost 100% of the rats studied, but bacterial translocation to distant organs (spleen and liver) did not take place in rats with less extended bowel resection and with intact ileocecal valve.
5. Based on the findings in this thesis it is difficult to conclude how far BT is vital for individual health. It might be regarded as a pathological finding without any serious side effect in an otherwise healthy and non-immunosuppressed individual. However, this is rarely the case in a host with ongoing BT often as a direct consequence of a severe coexisting disease such as SBS.
6. The absent of an ileocecal valve, treatment with gastric acid blocking medicines and gut decontamination medications were significantly associated with SBBO and subsequent BT
8. There seems to be a significant relationship between positive duodenal aspirates and cecal stool cultures and subsequent risk of bacteria translocation to distant organs. Massive intestinal resection is followed by increased number of patogenic bacteria in rats duodenal and cecal stool culture. Associated liver disease was noted in our study by the presence of progressive cholestasis, portal and lobular inflammation, macrophage hyperplasia, bile duct proliferation and fibrosis. Excess bacteria in the intestinal lumen caused deconjugation of bile salt acids which led to steatorrhea, these gradually progressed further to liver failure warranting animal euthanasia.
9. The malnutrition that followed extended bowel resection was another factor that further decreased gastric acidity and host immune function and exposed host to SBBO
10. There is an intricate ongoing struggle between the mucosal barrier and the intestinal microorganism which predisposes subject to BT
11. Strategies to increase adaptation in our study remain elusive despite an abundance of experimental data.
12. This study permits better prediction of short bowel syndrome outcomes which may help direct better future management of these challenging cases in human.