Early intestinal morphological changes following benzalkonium chloride treatment in a rat model of short bowel syndrome

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BACKGROUND: *The aim of this study was to assess early morphological changes of the residual small intestine 30 days after application of Benzalkonium Chloride (BAC) in a rat model of short bowel syndrome (SBS).*
MATERIALS AND METHODS: *Twenty nine Wistar rats (260 ± 20g) underwent 80% midsmall bowel resection with end to end anastomosis. In group 1 (n=14) BAC solution 0.1% was applied to a 2cm segment of jejunum, and in group 2 (n=15) normal saline was applied in a similar manner and the rats were sacrificed 30 days after operation. Specimens for histological examination were obtained initially and at sacrifice.*
RESULTS: *In the BAC treated jejunal segment (group 1), a statistically significant increase (p< 0.05) was noted in villous height by 33.2%, in crypt depth by 26.4%, in muscle thickness by 26%, 109.6% in intestinal diameter, and 20% in total intestinal length, compared to group 2.*
CONCLUSIONS: *BAC application to the serosal surface of rat's jejunum in SBS is a simple method that within only 4 weeks can topically augment the natural adaptation process noticed following intestinal resection. Further research with a tapering technique performed in sequence is suggested, to prevent possible problems associated with pseudoobstruction in the long term.*

KEYWORDS: Adaptation, Benzalkonium chloride, Intestinal resection, Short bowel syndrome.

Introduction

Short bowel syndrome (SBS) is the malabsorptive state that often follows extensive resection of the small intestine and continues to be a significant cause of morbidity and mortality. Certain patients may require lifelong total parenteral nutrition (TPN) depending on the length and health of the residual small bowel. Prolonged TPN administration though, is associated with certain complications, which include catheter sepsis and liver failure[1].

Surgery in patients with SBS should be performed with the primary intent of eliminating a patient's need for TPN and its associated complications. However, none of the up to date described operations are sufficiently safe and effective to recommend their routine use. For this reason many studies regarding the treatment of small bowel syndrome continue to be performed.

Intrinsic myenteric denervation of an intestinal segment with the cationic surfactant benzalkonium chloride (BAC) in experimental models of SBS is a recently described technique of improvement of this condition[2-4]

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that can be applied during the initial intestinal resection, without the need for additional anastomoses.
The aim of this study was to assess the intestinal morphological changes noticed in a period as early as 30 days after BAC treatment and discuss the significance that these may have in the acute phase of SBS, as well as our concerns about the possible long term effects of this technique.

Materials and methods

Twenty nine male Wistar rats (Pasteur Institution, Athens) with a mean body weight of 260 ± 20g were used. Procedures involving animals and their care were in conformity with institutional guidelines that comply with national and international laws and policies.
Body weight was measured before laparotomy and just before sacrifice at 4 weeks time. The animals were anaesthetized with an intraperitoneal injection of a solution containing ketamine (67mg/kg) and xylazine (13mg/kg). A prophylactic dose of cefamandole (30mg/kg) was also given intramuscularly at the beginning of the procedure. The animal's abdominal fur was shaved, the skin cleaned and disinfected with iodine solution and a 3cm midline incision was made. The distance between Treitz ligament and the ileocaecal valve was measured from the antimesenteric side without stretching the intestine and 80% midsmall bowel resection with end to end anastomosis was performed using 6/0 polyglactic acid. The remaining small intestine consisted of equal lengths of jejunum and ileum.
The animals were randomly assigned into 2 groups. In group 1 (n= 14) a 2.0cm segment of jejunum, 2.0cm proximal to the anastomosis was treated by topical application of 0.1% benzalkonium chloride solution (BAC), to the serosal surface for 30 minutes. Gauze was wrapped around the circumference of the test segment and BAC was applied above it, by an injector 6 times at 5 min intervals. During the application contamination of the other intestinal segments with BAC was prevented. In group 2 (n= 15), 0.9% normal saline was applied in a similar manner as a non drug treatment. After treatment, the serosa of the test segment was thoroughly rinsed with normal saline and returned to the peritoneal cavity in both groups.
During the operation and recovery from anesthesia rats were placed under a heating lamp to prevent hypothermia. Each animal at the end of the procedure received 5cc Ringer's solution subcutaneously and another dose of cefamandole (30mg/kg) intramuscularly. Animals were allowed to recover from anesthesia and given free access to water. Standard pellet chow was introduced after the first postoperative day, and a multivitamin was added to their water once a week.
The rats were sacrificed 1 month after the initial operation with an anesthetic overdose. Autopsy was performed and biopsies were taken from identical intestinal segments of jejunum, ileum at the initial operation, as well as from the treated jejunum and the same area proximally and distally of this, at the second laparotomy. Formalin-fixed specimens were embedded in paraffin and sections were stained with haematoxylin and eosin for histological evaluation by light microscopy (Zeiss Actiostar plus). Longitudinal sections were also cut for measurement of ganglia in the myenteric plexuses. Image analysis was then performed with the Axio Vision Image Analysis Software (Zeiss).

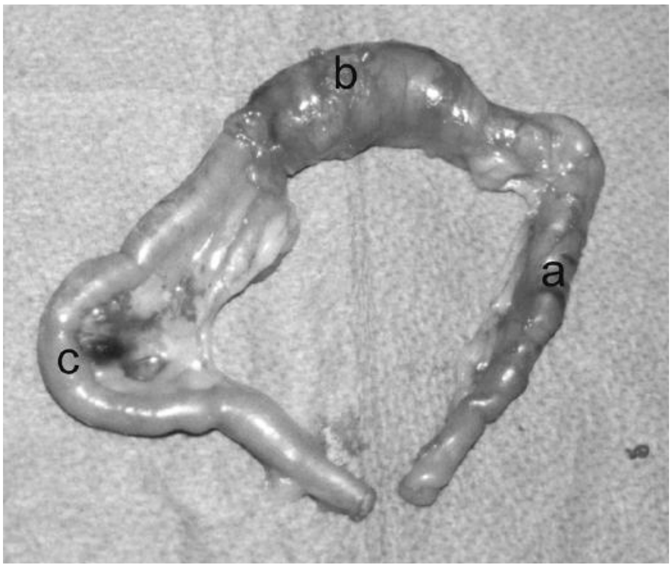

Fig. 1: Morphology of the remaining small bowel 4 weeks after extensive small bowel resection and BAC treatment. Enlargement of the BAC treated segment of jejunum (b), in comparison with the non treated jejunal segment (a) and ileum (c).

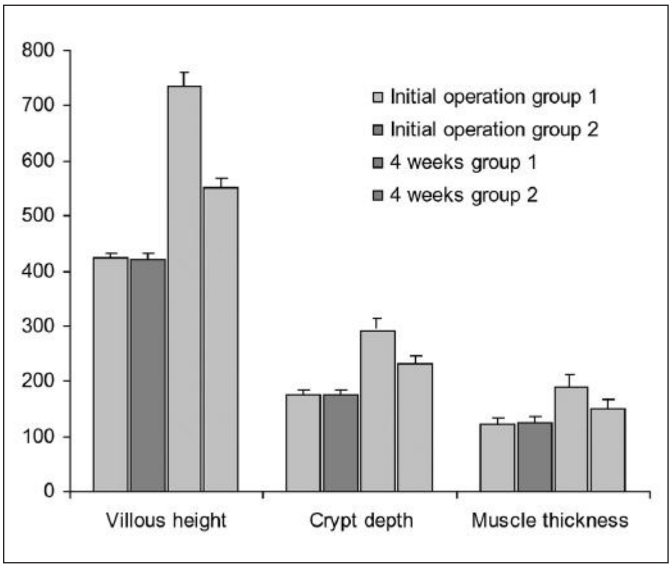

Fig. 2: Morphological changes of the treated jejunal segment with BAC (group 1) and N/Saline (group 2) 30 days postoperatively, compared to the initial biopsies. †$P<0.05$ (Unpaired Student's t test).

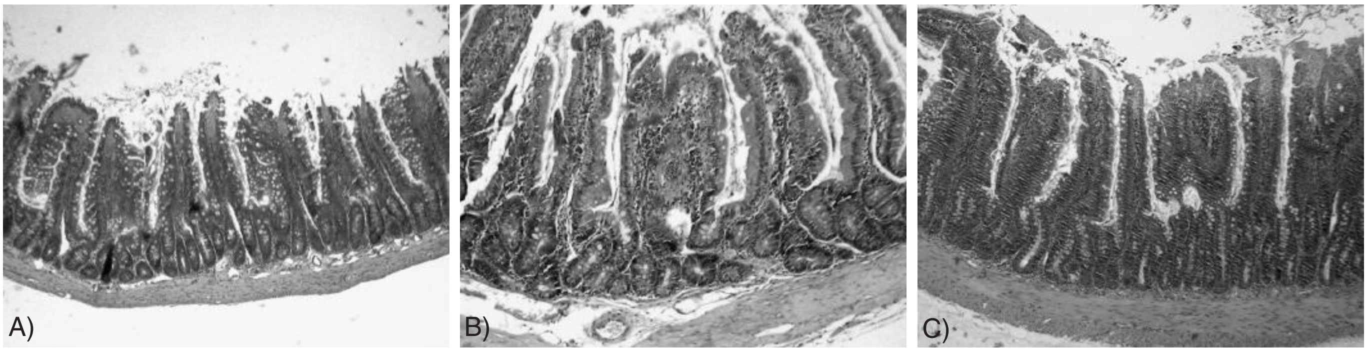

Fig. 3: Representative histological sections of jejunum. (a) Initial operation; (b) BAC treated segment (group 1), 4 weeks after the first laparotomy; (c) Resected group of animals (group 2), 4 weeks postoperatively. (Haematoxylin and eosin stain, original magnification X 60).

All values were expressed as mean ± standard deviation. Student's t test was performed for statistical analysis between the groups with $p<0.05$ considered statistically significant. SPSS 13 for Windows was used for statistical calculations.

Results

Thirty days after BAC treatment the number of ganglia in the myenteric plexus was reduced by 80% in the treated segment, from 19.40 ± 1.70 to 4.07 ± 1.20 per mm^2. In the denervated segment of jejunum (group 1) there was a statistically significant increase ($p< 0.05$) of 33.2% in villous height, 26.4% in crypt depth, 26% in muscular thickness and 109.6% in intestinal diameter (10.35 ± 0.70 vs. 4.93 ± 0.40 mm) compared to the control group (group 2) at that time (Figures 1, 2 and 3). A 20% increase in total small intestinal length was also documented 4 weeks postoperatively betweens groups 1 and 2 (36 ± 2 versus 30 ± 4 cm, $p< 0.05$). No statistically significant changes in intestinal morphology were noticed at the level of the non treated jejunum and ileum between the two groups 4 weeks postoperatively.
The mean body weight in group 1 (328 ± 19g) at the end of four weeks was significantly increased ($p= 0.006$) by 15% in comparison to the control group 2 (285 ± 31g).
Mortality was 14.2% in group 1 (2/14), and 20% in group 2(3/15). All deaths occurred within 12hrs postoperatively. One of the deaths in group 1 was associated with ether inhalation anesthesia just prior to the intraperitoneal injection. Necropsy revealed no evidence of perforation, anastomotic leak or obstruction in any of the animals.

Discussion

Massive intestinal resection triggers a morphological and functional adaptation of the remaining intestine through villous hyperplasia, intestinal dilatation and decreased gastrointestinal transit[5,6], often resulting in long-term dependence on TPN for many months or years. It is this subpopulation of patients that may benefit from surgery.
Among the procedures described to improve SBS are the construction of antiperistaltic segments[7-9] and valves[10-13], colonic interposition[14-16], small bowel lengthening[17], electrical pacing[18,19] and small bowel transplantation[20] which have proven to be difficult with high mortality.
Topical application of BAC on the serosal surface of the bowel has been reported to cause disruption of the myenteric plexus neurons [21-24]. BAC seems to induce irreversible depolarization and progressive degeneration of the cell membrane[25]. It has been suggested that the higher the level of the negative membranous charge, the stronger would be the injurious action of the cationic surfactant, explaining the selective injurious ablation in the nervous elements (-70mV to -90mV) compared to other tissues such as smooth muscle (-30mV to -70mV) [21]. Holle[26] and Hadzijahic[27] noticed significant increases of villous height, crypt depth and thickening of the musculature after BAC treatment, using different concentrations of BAC solutions each, in healthy rat models. It has also been demonstrated by Hadzijahic[27] that surgical denervation, where the myenteric plexus was removed with stripping of the serosa and longitudinal muscle from the intestinal wall, had a direct and identical effect on intestinal morphology as chemical ablation. Therefore the morphological changes noted are the result of intrinsic denervation of the myenteric plexus rather than a specific effect of BAC.
Fox et al[23] also documented that the basic electric rhythm (BER) and migrating myoelectric complex (MMC) of the treated with BAC segment of small bowel were disrupted. BER was slowed in the bowel distal to the site of denervation. Subsequent studies showed a return of the non-propagating MMCs after 13 to 17 days[24,28] but continued disruption of the propagating BER in the jejunal area devoid of myenteric neurons[24].
Chemically induced bowel denervation in a short bowel syndrome experimental model was first studied by Sawchuk et al 1987[2]. Their data demonstrated that treatment of a jejunal segment with BAC resulted in pro-

longed intestinal transit time, improved D-xylose absorption and weight gain in rats with short bowel syndrome. Garcia et al 1999[3] showed in accordance with Sawchuk, that intrinsic bowel denervation improves survival of rats with small bowel syndrome. A weight increase similar to that of the control animals was noted in the denervated group, as well as dilatation and increase in villous length of the denervated ileum 90 days after the initial procedure.

Sencan et al[4] using 0.1% BAC solution documented detailed changes in the intestinal morphology with statistically significant increases noticed in villous height, crypt depth, villous density, muscular thickness and diameter of the denervated jejunum 3 months postoperatively.

BAC application in our study also seems to augment, topically where applied, the structural intestinal adaptation that would have normally been noticed after extensive small bowel resection, only 4 weeks after the initial operation in the treated animals. This theoretically may correspond to earlier reduction of TPN and introduction of enteral nutrition at an early stage which is a key factor in achieving intestinal autonomy in patients with SBS.

Following intestinal resection sufficient to cause SBS, the residual intestine in the long term may become dilated and dysmotile, leading to stasis of intestinal nutrients, thereby promoting small bowel bacterial overgrowth (SBBO) as well as increased translocation of intestinally derived microorganisms and their toxic products. These in turn cause infectious complications, cholestasis and progressive liver disease[29,30]. The creation of an aganglionic segment, causing further dilatation and decreased intestinal motility, raises concerns about exacerbation of these problems.

Pseudoobstruction is commonly detected by contrast radiological studies such as a barium follow through investigation which should be routinely performed in the assessment of patients with prolonged intestinal failure. In this situation a tapering enteroplasty of the dilated bowel segment could be performed to prevent problems associated with a grossly dilated residual intestine. New techniques described such as the serial transverse enteroplasty (STEP procedure) appear to be more efficient than conventional bowel lengthening methods, and can theoretically increase the intestinal length more than double compared to the Bianchi procedure[31-33].

In conclusion, chemical denervation of a jejunal segment with BAC is a promising technique of increasing the absorptive surface in the acute phase of short bowel syndrome. However there are concerns about exacerbation of problems associated with pseudoobstruction in the long term, where a tapering operation can then be performed, if that occurs. Further research in porcine models should be promoted so that the described method with possibly the addition of a tapering operation, can henceforth find application in clinical practice.

Riassunto

Obiettivo: Lo scopo dello studio è di valutare I cambiamenti morfologici precoci dell'intestino tenue, 30 giorni dopo l'applicazione di cloruro di Benzalconio (BAC) in un modelo di ratto con sindrome dell'intestino corto (SBS). In aggiunta ci sono esaminate morfologia e funzione epatica.

Materiali e Metodi: 29 ratti Wistar erano sottoposti a resezione centrale del 80% dell'intestino tenue e biopsia epatica. Nel gruppo 1 (n= 14) è stata applicata in un segmento di 2 cm del digiuno la soluzione BAC 0.1%, e nel gruppo 2 (n= 15) è stata applicata in maniera simile soluzione salina 0.9%. I campioni per l'esame istologico e l'analisi di funzione epatica, sono stati ottenuti al momento dell'intervento e dopo quattro settimane.

Risultati: Nel primo gruppo trattato con BAC, è stato osservato, un aumento statisticamente significante (p-<0.0001) dell'altezza dei vili di 32.2%, della profondità delle cripte di 26.4%, dello spessore muscolare di 26% e del diametro intestinale di 109.6%, in confronto con il gruppo 2. L'esame istologico non ha rilevato steatosi o cirrosi in nessun animale.

Conclusioni: L'applicazione del BAC sulla superficie serosa digiunale del ratto con SBS costituisce un metodo semplice ed efficace che in solo quattro settimane può incrementare il processo naturale dell'adattamento dopo resezione intestinale.

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