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CHAPTER 1

Intestinal barrier function in patients undergoing (sub)total colectomy

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Abstract

Aim

Aim of this pilot study was to determine whether the type of approach, open or laparoscopic, and the order of devascularisation in laparoscopic colectomy, affects intestinal barrier function, local inflammatory response and clinical outcome.

Method

Elective colectomy patients were included from April 2006 to July 2008. After informed consent, 22 patients scheduled for laparoscopic colectomy were randomized to start with inferior mesenteric artery or ileocolic artery devascularisation. Eighteen patients scheduled for open surgery served as a prospective control group. To assess the intestinal barrier function release of intestinal fatty acid binding protein (I-FABP; marker of mucosal injury and ischemia) was measured pre- and postoperatively. Mesenteric lymph nodes were harvested to assess expression of inflammatory mediator-related genes using Multiplex Ligation Probe Amplification. The study was registered under NTR1025.

Results

Laparoscopic devascularisation started at the ileocolic artery resulted in a significantly increased excretion of I-FABP over time (P=0.002). In this group I-FABP levels were significantly increased on postoperative days 1 and 3 compared to pre-operative values (P=0.011 and P=0.001, respectively). There were no differences in expression of inflammatory mediator-related genes or postoperative morbidity among the groups.

Conclusions

In this pilot study it was demonstrated that devascularisation started at the ileocolic artery during laparoscopic colectomy was associated with prolonged intestinal mucosal ischemia.

Introduction

The lumen of the large bowel contains around 10^{12} bacteria per mL of faeces.[1] Under normal circumstances, the gastrointestinal tract has the ability to separate these potentially pathogenic bacteria and other products from the extraluminal environment. This is the so called 'intestinal barrier function' and its function can be compromised by infectious and inflammatory conditions, cytotoxic drugs, radiation therapy, thermal injury, stress and ischemia.[2] Failure of the intestinal barrier function enhances intestinal permeability and can lead to Bacterial Translocation (BT). BT is the passage of bacteria across the intestinal epithelium to sterile extraintestinal sites, such as mesenteric lymph nodes (MLNs) and other organs and forms the basis of the 'gut origin of sepsis' hypothesis.[3-5] There is no consensus about the clinical and pathophysiological significance of BT.[6] However, a recent study including 927 patients over 13 years showed that BT was associated with increased postoperative septic morbidity in surgical patients.[7]

The most reliable method to evaluate BT is by sampling and culturing MLNs, in which a positive culture indicates BT.[8] BT can lead to reaction in the mesenteric immune system resulting in up- or down regulation of inflammation-related genes. Multiplex Ligation robe Amplification (MLPA) can detect changes in RNA expression of genes encoding for inflammatory mediators.[9]

As stated before, the 'intestinal barrier function' can be compromised by ischemia. Nowadays, there are several serological markers available for the early diagnosis of intestinal ischemia, for example D-lactate, gluthathione S-transferase, and intestinal fatty acid binding protein (I-FABP). In this study intestinal ischemia was assessed by measuring I-FABP excretion in the urine. I-FABPs are small and abundant proteins within the cytoplasm of mature enterocytes located at the villus tip, the area most vulnerable to ischemia. A higher excretion of I-FABP correlates positively with intestinal ischemia.[10;11] A recently published systematic review has pooled the results of 3 studies to calculate diagnostic accuracy. They stated a sensitivity of 72% (51%-88%) and a

specificity of 73% (62%-83%).[12]

In laparoscopic surgery mostly a medial to lateral approach is applied, particularly in colorectal cancer, instead of the more commonly used lateral to medial approach in open surgery. In the medial to lateral approach vessel ligation is the first step in the procedure while in the lateral to medial approach vessel ligation is one of the last steps in the procedure. The medial to lateral approach results in ischemia in the beginning of the procedure, since parts of the colon will be devascularised at an earlier stage than in the open procedure. It can be hypothesized that this situation leads to more BT.

In laparoscopic colectomy devascularisation can be started on the left side at the inferior mesenteric artery (IMA) or at the right side at the ileocolic artery (ICA). When devascularisation is started at IMA, large bowel ischemia might be less pronounced because of the presence of collateral flow from ileocolic vessels and the superior rectal artery. Devascularisation started at ICA results in more rapid progression of colonic ischemia as there is no collateral flow from the terminal ileum. Apart from the difference in approach and order of devascularisation the operating time for a laparoscopic colectomy is longer. This prolonged operating time as well as the time of bowel ischemia may lead to progressive BT.

The aim of this pilot study was to determine whether the type of approach, open or laparoscopic, and the order of devascularisation in laparoscopic colectomy, affects intestinal barrier function, local inflammatory response and clinical outcome.

Materials and methods

Patients undergoing laparoscopic or open proctocolectomy with ileal pouch-anal anastomosis or laparoscopic or open (sub)total colectomy with ileal rectal anastomosis for inflammatory bowel disease, familial adenomatous polyposis, hereditary nonpolyposis colorectal cancer or colorectal malignancy were eligible for this study. Exclusion criteria were: patients under 18 years, no informed consent or antibiotics within a week prior to surgery. This study was approved by the Medical Ethics Committee of the Academic Medical Center (Amsterdam, The Netherlands) and registered under NTR1025.

Patients were preoperatively assigned to an open or laparoscopic procedure on a case by case basis by the operating surgeon. Patients planned for a laparoscopic resection were randomly assigned to start at the IMA or ICA with devascularisation using sealed nontransparent envelopes. Patients who underwent an open procedure served as prospective control group.

Primary endpoints were: excretion of intestinal fatty acid binding protein (I-FABP) in urine preoperatively and at postoperative days 1, 3, and 7, and expression of in

flammatory mediator-related genes in mesenteric lymph nodes (MLNs). Secondary endpoints were: overall morbidity, number of reoperations, readmission rate, primary and total hospital stay and mortality rate. Morbidity was defined as any complication requiring unplanned medical or surgical intervention within 30 days surgery. Total hospital stay was defined as primary hospital stay plus the hospitalization period of patients who were readmitted within 30 days after surgery.

Surgical technique

In an open colectomy right and left flexures were completely mobilized before ligating arteries and venes. In the left-sided laparoscopic approach, the IMA was ligated first, followed by the left and right branch of the medial colic artery and finally the ICA. The left hemicolon is not completely devascularised until the pelvic phase of the operation due to collateral flow from the rectum and terminal ileum. In the right sided approach, ICA was ligated first followed by the branches of the middle colic artery, resulting in a completely devascularised right hemicolon. In a later phase, the IMA and inferior mesenteric vein were ligated.

Intestinal fatty acid binding protein (I-FABP) in urine

I-FABP is a marker of mucosal injury and ischemia, and excretion in the urine increases if intestinal ischemia occurs.[11;12;14;15] I-FABP was assayed in urine collected for 12 hours at the day before operation and at postoperative days 1, 3, and 7. At day 7, urine was only sampled if the patient was still in the hospital. Two mL of the homogenized urine was stored at -20 °C until analysis. Determination of I-FABP concentration was performed by enzyme-linked immunosorbent assay. A commercially available kit (Hycult Biotechnology b.v. Uden, The Netherlands) was used, and the assay performed in accordance with the manufacturer's instructions. The detection limit was 20 pg/mL. The concentration of I-FABP excretion measured in the 2 mL of the homogenized urine was multiplied by the 12-hour urine volume. This was done to calculate the total amount of I-FABP excreted in 12 hours.

Multiplex Ligation Probe Amplification (MLPA) in mesenterial lymph nodes (MLNs)

MLNs were sampled to asses expression of inflammatory mediator-related genes using MLPA. At the end of the operation, after specimen retrieval, a lymph node from the distal ileum mesentery was sampled and cut into two parts in a sterile area with sterile surgical instruments, put into sterile numbered tubes and stored at -80 °C. To isolate RNA, frozen lymph nodes were homogenized in liquid nitrogen using a mortar and pestle and a commercial RNA extraction kit (Nucleospin® RNA II, Macherey-Nagel GmbH & Co., KG Düren, Germany). After completion of the protocol total RNA was dissolved in 60 µL RNase-free water and stored at -80 °C until further analysis. Changes in RNA expression of interleukins and cytokines (IL15-R01, IL18, IL18b,

IL1RN, IL2, IL6, IL10, ScyA2, ScyA3, ScyA4, ScyA8) enzyme and enzyme inhibitors (CDKN1a, PARN, PDE4B, GSTP1, PTPN1, PTP4A2, SerpinB9) transcription factors and oncogenes (BMI1, MYC, NFkB2, NFkBIA, NFkB1) and other cellular factors (THBS1, LTA, Tnfrsf1a, MIF, PDGFb, TF, TNF) in the MLNs were assayed using MLPA human inflammation kits (R009, MRC-Holland, Amsterdam, The Netherlands) and expressed relative to the household gene Beta-2-Microglobulin (B2M). The assays were performed according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using SPSS for Windows version 15.0.1. All data for this pilot study were presented as median (inter-quartile range). For dichotomous endpoints, treatment groups were compared by using Chi-square test where appropriate. Mann Whitney U test and Kruskal Wallis test were used for quantitative endpoints when comparing two or more groups. The distribution of data over time between groups was analyzed using repeated measures with non-parametric ANOVA. The distribution of data per group per day was analyzed using non-parametric ANOVA. All values were rank transformed and to adjust for confounding effects IBD was introduced as a covariate. Significance was set at $P<0.05$, with appropriate Bonferroni corrections for multiple comparisons being employed in post hoc tests.

Results

Between April 2006 and July 2008, 84 patients were eligible of whom 40 (48%) gave informed consent. Of the included patients 18 underwent an open and 22 patients a laparoscopic operation. Of the laparoscopic group, 11 patients were randomized to start devascularisation at IMA and 11 to start at ICA. Apart from age distribution and operation time, there were no significant differences in patient characteristics (Table 1).

Table 1 Patient characteristics

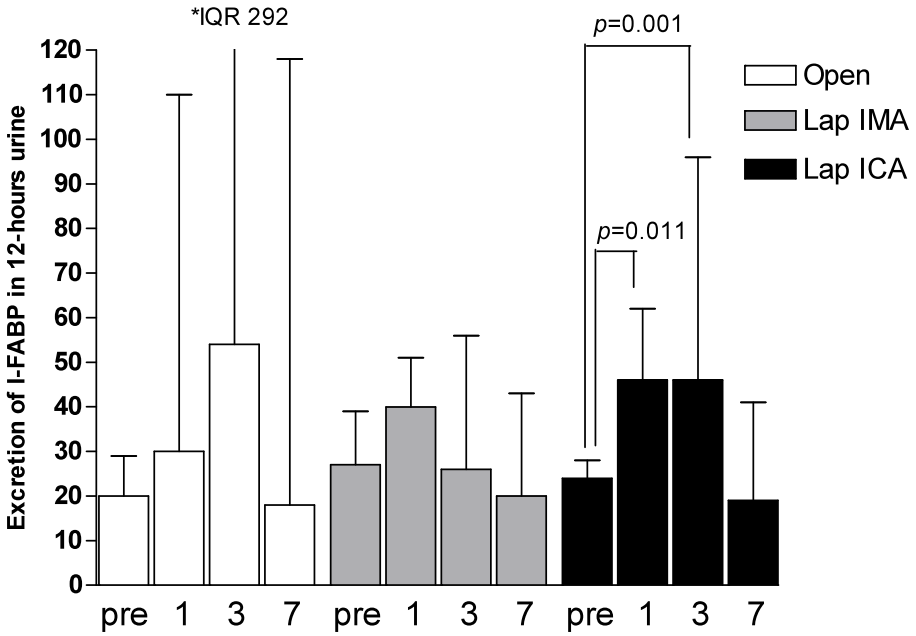
	Open (sub)total colectomy	IMA start laparoscopic (sub)total colectomy	ICA start laparoscopic (sub)total colectomy	P
N	18	11	11	
Male : female	14 : 4	6 : 5	7 : 4	0.410$^{\pm}$
Age	61 (38-68)	31 (24-42)	29 (23-36)	0.004†
ASA I : II : III	6 : 8 : 4	7 : 4 : 0	5 : 6 : 0	0.153$^{\pm}$
Body Mass Index (kg/m^2)	24.2 (22-26)	22.8 (22-24)	22.3 (21-27)	0.545†
Operating time	166 (145-191)	293 (258-298)	280 (264-323)	<0.001†
Diagnosis				0.537$^{\pm}$
- IBD	9	7	6	
- FAP	5	4	4	
- HNPCC	1	0	1	
- Colorectal malignancy	3	0	0	
Type of surgery				0.302$^{\pm}$
- IPAA	13	10	10	
- IRA	5	1	1	

Values are median (inter-quartile range) / IMA = inferior mesenteric artery / ICA = ileocolic artery / ASA = American Society of Anaesthesiologists / IBD = Inflammatory Bowel Disease / FAP = Familial Adenomatous Polyposis / HNPCC = Hereditary Nonpolyposis Colorectal Cancer / IPAA = proctocolectomy and ileal-pouch anal anastomosis / IRA = (sub)total colectomy and ileal-rectal anastomosis / $^{\pm}$Chi-square test / †Kruskal-Wallis test

Measurement of mucosal injury and ischemia: I-FABP excretion in urine

I-FABP excretion was similar when compared on a day by day basis. When analyzing I-FABP excretion over time; a right-sided (ICA) start of devascularisation resulted in significantly increased excretion of I-FABP over time (P=0.002; Figure 1). I-FABP levels were significantly increased on postoperative days 1 and 3 compared to preoperative values (P=0.011 and P=0.001, respectively; Figure 1). After an open colectomy or a left-sided (IMA) start there was no significant effect on the excretion of I-FABP over time (P=0.111 and P=0.531, respectively).

Figure 1 Excretion of I-FABP levels in 12-hour urine preoperatively (pre), postoperative day 1 (1), postoperative day 3 (3) and postoperative day 7 (7) for patients undergoing an open, laparoscopic IMA or laparoscopic ICA (sub)total colectomy



Values are median and inter-quartile range / Repeated measures with non-parametric ANOVA, pairwise comparisons / I-FABP excretion over time, P=0.002

Patients with colonic inflammatory bowel disease were compared to the non-inflammatory diseases needing surgery. There was no significant difference in I-FABP excretion in urine on any of the postoperative days between patients operated for inflammatory bowel disease (n=22) compared to those operated for non-inflammatory bowel disease indication (n=18). Moreover, there was no significant difference in I-FABP excretion in urine on any of the postoperative days between patients with or without morbidity.

Measurement of inflammatory mediator-related genes: MLPA

MLPA showed, after post hoc correction for multiple testing (P<0.0017), no statistical differences in RNA expression for inflammatory mediator-related genes between an open or laparoscopic IMA or ICA approach. Neither for patients operated for inflammatory bowel disease (n=22) or operated for non-inflammatory bowel disease

indication (n=18).

Postoperative results

None of the laparoscopic procedures were converted. There were no differences between the groups in secondary endpoints (Table 2).

Table 2 Postoperative results

	Open (sub)total colectomy	IMA start laparoscopic (sub)total colectomy	ICA start laparoscopic (sub)total colectomy	P
N	18	11	11	
No. of patients with morbidity within 30 days	7	1	2	0.165±
Reoperation	5	1	2	0.467±
Readmission within 30 days	1	1	2	0.542±
Primary hospital stay (days)	9 (7-15)	10 (7-11)	8 (6-8)	0.220†
Total hospital stay (days)	9 (7-22)	10 (7-13)	8 (7-12)	0.424†
Mortality within 30 days	0	0	0	

Values are median (inter-quartile range) / IMA = inferior mesenteric artery / ICA = ileocolic artery / ±Chi-square test / †Kruskal-Wallis test

Discussion

The present pilot study indicated that devascularisation started at the ICA during laparoscopic colectomy was associated with prolonged intestinal mucosal ischemia. However, neither the type of approach, open or laparoscopic, nor the order of devascularisation in laparoscopic colectomy, affected the local inflammatory response and clinical outcome. The fear of some laparoscopic surgeons that this would be a disadvantage of the medial to lateral approach is therefore not justified.

In this study patients that underwent open surgery were significantly older. Nevertheless, we do not think that this compromised the comparability between the groups with respect to primary endpoints. There was also some disparity in number of patients that underwent IRA between the open and laparoscopic groups. The difference between IRA and IPAA is that with an IRA the rectum does not need to be extirpated. As during the rectal extirpation phase of the operation, the devascularised colon has already been removed, this is not of influence on the outcome.

The high complication rate is remarkable in the open group, this can be explained by the fact that all complications, both intra- and extramural, were scored prospectively.

Apart from the order of devascularisation, difference in operating time between the three groups might have influenced the results. Obviously, operating time of an open approach was significantly shorter than of a laparoscopic approach. The colectomy part in the laparoscopic approach, i.e. the time the devascularised bowel was still in connection with the circulation, was 2-2.5 hours, while total operating time was more than 4 hour. Devascularisation time of the colectomy may have been too short to produce significant clinical inflammatory response.

Unfortunately, this study did not show any statistical clinical differences. There are several explanations for the discrepancy between the significant increase in I-FABP and the similar clinical outcomes. First of all, we did not perform a power calculation and therefore this was an underpowered study. Secondly, all laparoscopic operations have been performed by a fellow surgeon supervised by a senior surgeon (W.A.B.), and had normal operation times. Thirdly, patients were electively operated. This indicates that patients were not very ill. In our opinion, clinical relevance might show, if duration of the colectomy is prolonged, for example when in the hands of a surgeon still in its learning curve or when the operation is more complex or in an acute setting. Therefore, when long operating times are to be expected, devascularisation starting at IMA during laparoscopic colectomy may be preferred.

The inflammatory response caused by BT was assessed in sampled MLNs using MLPA. MLPA is a new method to detect changes in RNA expression of inflammation-related genes. In the present study, there were no significant changes in RNA expression of inflammatory mediator-related genes. It may well be that antibiotics prophylaxis given at the start of the operation affected the extent of this response. Although it is not likely that one dose of antibiotics changed luminal contents that much, but this cannot be ruled out. For example, pseudomembraneous colitis has been described after operative antibiotics prophylaxis.[16]

Limitations of our study were the small sample size, the heterogeneity of the illness of the patient, the and non-randomized study design of the open group.

In conclusion, the present study indicated no differences in outcome measures, except that devascularisation started at the ICA during laparoscopic colectomy was associated with prolonged intestinal mucosal ischemia.

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