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ORIGINAL ARTICLE

Pro-inflammatory Cytokine Release in Rectal Surgery: Comparison Between Laparoscopic and Open Surgical Techniques

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Abstract The objective of the present study was to investigate whether laparoscopic rectal surgery causes a less pronounced release of pro-inflammatory cytokines as compared to open surgical technique. Twenty-four consecutive patients undergoing rectal surgery due to cancer disease were included in a prospective and randomized trial. The patients were randomized to laparoscopic ($n = 12$) or open surgery ($n = 12$). Blood was sampled at five occasions; after induction of anesthesia before start of surgery, at 180, 360 min and 24 h after start of surgery and the last sample was taken in the late post-operative period 3–5 days after surgery. The levels of interleukin (IL)-1α, IL-6, IL-8, IL-10, tumor necrosis factor-α, C-reactive protein (CRP), white blood cells, intracellular adhesion molecule-1 and vascular cell adhesion molecule-1 were analyzed using multiplex sandwich enzyme-linked immunosorbent assay. There was a release of both pro- and anti-inflammatory cytokines during colorectal surgery. The release of IL-6, IL-10 and CRP was significantly lower in the laparoscopic group. Rectal surgery causes release of both pro- and anti-inflammatory cytokines. The inflammatory response is lower in laparoscopic rectal surgery as compared to conventional open surgery. Less tissue trauma in laparoscopic rectal surgery and/or less peri-operative bleeding in the laparoscopic cases leads to a lower degree of inflammatory response.

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Introduction

Surgical trauma causes a host inflammatory reaction (Lenz et al. 2007). Pro- and anti-inflammatory cytokines are released and the systemic inflammatory response syndrome (SIRS) can result with tachycardia, tachypnea, leukocytosis and fever (Bone et al. 1992; Herzum and Renz 2008). In its most severe form, the systemic inflammation can cause failure of one or several vital organs (Dewar et al. 2009). Multiple organ dysfunction syndrome (MODS) can be the consequence after trauma with severe systemic inflammation. It is therefore of interest to find surgical techniques that are less traumatic and causes less systemic inflammation. It has been shown in previous studies that laparoscopic surgery preserves immune function better than conventional open surgery and is associated with less infectious complications (Bilimoria et al. 2008; Buunen et al. 2004). Colorectal cancer causes increasing immunosuppression as the disease progresses (Evans et al. 2010). It might, therefore, be an oncological advantage to use a surgical technique with less immunological impact.

C-reactive protein (CRP) is perhaps the most well-known acute phase reactant and the use of CRP is widely spread in clinical practice. The plasma concentration of CRP rapidly increases during bacterial infections, cancer and after tissue trauma (Clyne and Olshaker 1999; Zimmerman et al. 2003). IL-6 is a cytokine with both pro- and anti-inflammatory abilities and it is involved in the stress response to trauma, burns and sepsis (Jawa et al. 2011). Plasma interleukin (IL)-6 levels are elevated after surgical trauma and may predict surgical complications that result

in peritonitis (Yamamoto et al. 2011). Tumor necrosis factor (TNF)-α and IL-1α are both pro-inflammatory and induce the acute phase reaction with local and systemic inflammation (Aggarwal et al. 2006). IL-8 is a pro-inflammatory interleukin which is associated with growth and migration of tumor cells in colorectal cancer and angiogenesis (Ning et al. 2011).

IL-10 is an anti-inflammatory interleukin which stimulates the production of antibodies (Moore et al. 2001). An elevated plasma concentration of IL-10 before surgery has been associated with tumor recurrence in colon cancer patients (Galizia et al. 2002). Intracellular adhesion molecule-1 (ICAM-1) is involved in recruitment of leukocytes from the circulation to the site of infection. Leukocytes adhere to ICAM-1 molecules on the vascular endothelial cells and then migrate through the vessel wall (Rijcken et al. 2002). Vascular cell adhesion molecule-1 (VCAM-1) is also involved in cell adhesion to the endothelium. VCAM-1 is upregulated after stimulation by TNF-α and IL-1α and mediates adhesion by monocytes, lymphocytes, eosinophils and basophils (Chavakis et al. 2009). Also other cascade reactions occur such as activation of complement (Bengtsson 1993; Flierl et al. 2006).

The objective of the present study was to investigate whether laparoscopic rectal surgery causes a less pronounced release of pro-inflammatory cytokines compared to open surgical technique.

Materials and Methods

The present study is a prospective and randomized trial. The study was performed in a university hospital in western Sweden. Twenty-four patients were included in the study. The patients were subject to anterior rectal resection or abdomino-perineal resection due to rectal cancer. The patients were randomized to either open or laparoscopic surgery. The patients were informed and gave their written consent according to the principles stated in the declaration of Helsinki. The study was approved by the ethical review board at Sahlgrenska University Hospital.

Before anesthesia was induced all patients received an arterial line in their left radial artery for blood sampling and continuous monitoring of blood pressure. The patients were also provided with an epidural catheter which was activated at the end of surgery for post-operative analgesia. Anesthesia was induced by administration of propofol (Diprivan®, AstraZeneca AB, Södertälje, Sweden). Before endotracheal intubation the patients received fentanyl (Leptanal®, Janssen-Cilag AB, Sollentuna, Sweden) and rocuronium (Esmeron®, Organon AB, Göteborg, Sweden). Ringer acetate solution was given at 4 ml/kg/h. Colloid fluids were given upon clinical consideration by the anesthetist. Blood was sampled at five different occasions. The first sample (T0) was taken after induction of anesthesia before start of surgery. The second sample (T1) was taken 180 min after start of surgery. The third sample (T2) was taken 360 min after start of surgery. Twenty-four hours after start of surgery the fourth sample was taken (T3). The final sample (T4) was taken in the late post-operative period 3–5 days post surgery. Blood-samples were collected in tubes coated with ethylene diamine tetraacetic acid (EDTA); 0.34 M EDTA per 4.5 ml blood. After centrifugation for removal of cells the samples were frozen within 30 min and stored at −80 °C.

The patients were operated on by surgeons in the same colorectal department. Surgeons were assigned according to availability. The laparoscopic team consisted of four surgeons. At least one of them was responsible for each laparoscopic operation. Open surgery was done through a midline incision from the pubis to about 10 cm above the umbilicus in both anterior (AR) and abdomino-perineal (APR) resection. In addition, a round perineal excision with the diameter of 5–10 cm was made in the APR. Laparoscopic surgery (AR and APR) was done through two–three 12-mm ports and one–two 5-mm ports. The specimen was extracted through a 6–8 cm long abdominal incision (AR) or an oval-shaped perineal excision with a diameter of 5–10 cm (APR). Intraabdominal pressure was kept between 9 and 12 mmHg. Conversion was defined as an abdominal incision measuring more than 8 cm.

The levels of IL-1α, IL-6, IL-8, IL-10, TNF-α, ICAM-1 and VCAM-1 were obtained with SearchLight method (Pierce Biotechnology, Woburn, MA, USA) which is a multiplex sandwich enzyme-linked immunosorbent assay in a planar, plate-based array format, for the quantitative measurement of secreted proteins in different biological materials. Diluted samples and controls were incubated for 1 h on the arrayed plates. All incubations were performed at room temperature with shaking at 200 rpm. The plates were decanted and washed six times before adding a cocktail of biotinylated detection antibodies to each well. After incubating with detection antibodies for 30 min, the plates were washed three times and incubated for 30 min with streptavidin-horseradish peroxidase. The plates were again washed before adding SuperSignal Femto Chemiluminescent substrate. The plates were immediately imaged using the Searchlight Black Ice imaging system, and data were analyzed using Array Analyst software (Auchon Biosystems, Billerica, MA, USA). All results were in the range of the standard curve. The levels of CRP and white blood cells were measured at the hospital laboratory for clinical chemistry.

All exploratory and formal statistical tests were carried out using SPSS for Windows (Version 18, SPSS Inc, Chicago, IL, USA). The repeated measures of continuous

data from each patient were analyzed using linear mixed models with type of surgery, time and the interaction term as fixed factors. Statistical significance was determined with p values less than 0.05. To estimate the sample size required, a power analysis was performed. The hypothesis was based on an expected 30 % lower activation rate of IL-6 and IL-8 in the laparoscopic group compared to the open surgical group. This was based on a significance criterion of 0.05.

Results

Table 1 shows the clinical characteristics of each surgery group. There was no significant difference between groups regarding age, sex, type of procedure (anterior resection or abdomino-perineal resection) or length of hospital stay post-operatively. There was a difference regarding peri-operative bleeding, duration of surgery and anesthesia. In the laparoscopic group the median bleeding was 200 and 1,150 ml in the open group. The median duration of surgery was 340 min in the laparoscopic group and 250 min in the open group. The median duration of anesthesia was 432 min in the laparoscopic group and 320 min in the open group (Table 1).

Table 2 shows the medians and inter quartile ranges for each cytokine by type of surgery and for each time point.

IL-6

Concentrations of IL-6 were significantly higher in the open group as compared to the laparoscopic group at T1 and T2. The mixed model analysis confirms a significant interaction effect between time and type of surgery ($p = 0.003$).

IL-8

The concentration of IL-8 is elevated compared to baseline but lower in the laparoscopic group. The mixed model analysis does however not confirm a significant interaction effect between time and type of surgery ($p = 0.069$).

IL-10

The mixed model analysis of data confirms a difference between groups. There is a significant interaction effect between time and type of surgery ($p = 0.008$). The difference between IL-10 concentrations was greatest at T1.

CRP

The median concentration of CRP in both groups at baseline was 5 mg/l. The concentration then increased in both groups. At T3, 24 h after surgery started, the median concentration was 57.5 mg/l in the laparoscopic group and 100.0 mg/l for the open group. At 3–5 days post surgery (T4) the concentrations were 60.0 mg/l in the laparoscopic group and 49.0 mg/l in the open group. The statistical analysis showed a significant interaction between type of surgery and time ($p = 0.012$).

Discussion

The present study shows that both pro- and anti-inflammatory cytokines are released during laparoscopic and open rectal surgery. The study also shows that there is a significantly lower release of IL-6, IL-10 and CRP in patients operated on with laparoscopic technique compared to open surgery during the operative period. Our findings of lower release of IL-6 in laparoscopic compared to open colorectal surgery confirm results from other studies (Sammour et al. 2010). In a recent randomized controlled trial by Pascual and colleagues (2011), lower IL-6 levels were measured in laparoscopic colectomy as compared to open surgery. The concentration of IL-6 is also elevated when complications occur such as post-operative sepsis and anastomotic leakage (Fouda et al. 2011; Mokart et al. 2005).

It has been suggested that the lesser degree of inflammatory response in laparoscopic surgery could have a positive oncologic effect with improved survival (Novitsky et al. 2004). Studies by Lacy et al. (2008) on laparoscopic vs open colectomy due to cancer shows a better long-term survival in the laparoscopic group. In colorectal cancer the

Table 1 Clinical characteristics of treatment groups

	Laparoscopic	Open	p value[1]
Age	65 (59–73)	61 (57–66)	0.478
Gender (F/M)	6/6	5/7	1.000
Anterior resection/abdomino-perineal resection (patients)	9/3	7/5	0.33
Duration of surgery (min)	340 (267–375)	250 (222–327)	0.033
Duration of anesthesia (min)	432 (375–472)	320 (272–402)	0.004
Blood loss (ml)	200 (100–288)	1,150 (425–1,500)	<0.001
Post-operative stay (days)	8 (5–10)	9 (6–11)	0.478

Values are given as median (25th–75th percentile)

[1] Mann–Whitney U test used for age, duration and blood loss. Fisher's exact test used for categorical data

Table 2 Median concentrations of inflammatory variables by type of surgery and timepoint

Inflammatory marker	Type of surgery	T0 0 min	T1 180 min	T2 360 min	T3 24 h	T4 3–5 days
IL-1α pg/ml (<1.1)*	Laparoscopic	0.8 (0.8–0.8)	0.8 (0.8–0.8)	0.8 (0.8–0.8)	0.8 (0.8–0.9)	0.8 (0.8–0.8)
	Open	0.9 (0.8–4.6)	0.8 (0.8–4.6)	0.8 (0.8–4.0)	0.8 (0.8–1.2)	0.8 (0.8–2.7)
IL-6 pg/ml (<5)*	Laparoscopic	4.7 (2.6–9.2)	**45.6 (29.3–69.1)**	**146.4 (106.0–200.1)**	156.9 (80.2–253.8)	30.9 (21.2–48.0)
	Open	4.7 (2.9–23.1)	**276.8 (176.2–409.2)**	**208.5 (145.5–412.2)**	144.1 (28.0–303.7)	43.4 (17.3–61.2)
IL-8 pg/ml (<70)*	Laparoscopic	**7.1 (5.2–10.9)**	10 (8.7–15.9)	15 (11.7–20.2)	20.6 (12.9–40.0)	12.5 (7.1–16.9)
	Open	**12.1 (9.1–16.3)**	23.6 (13.2–39.4)	28.9 (15.7–74.1)	18 (12.5–32.3)	16.7 (7.8–19.5)
IL-10 pg/ml (< 5)*	Laparoscopic	1 (0.5–1.9)	**2.8 (1.9–3.9)**	6.3 (4.2–19.7)	3.7 (1.9–7.2)	1.7 (1.2–2.2)
	Open	0.9 (0.6–2.2)	**11.2 (8.1–17.2)**	7.8 (3.3–16.3)	3.6 (1.7–4.6)	2.1 (1.6–3.1)
TNF-α pg/ml (<20)*	Laparoscopic	2.9 (1.7–14.2)	4.8 (2.5–16.2)	4.2 (2.8–11.0)	4.4 (3.2–15.4)	3.8 (2.8–22.4)
	Open	3.3 (2.1–8.9)	7.5 (3.5–11.4)	6.6 (3.3–16.4)	5.4 (3.3–10.9)	4.7 (2.3–11.9)
CRP ug/ml (<5)*	Laparoscopic	5 (5.0–5.0)	§	§	**57.5 (49.8–69.0)**	60 (28.0–75.0)
	Open	5 (5.0–6.5)	§	§	**100 (61.8–137.5)**	49 (30.5–147.5)
WBC × 10^9/ml (3.5–8.8)*	Laparoscopic	3.7 (3.2–5.9)	§	§	8.3 (6.1–8.9)	6.8 (5.5–7.6)
	Open	3.5 (2.8–4.8)	§	§	6.8 (5.1–8.8)	6.9 (3.4–8.9)
ICAM-1 ng/ml (<1,100)*	Laparoscopic	222 (187–345)	237 (192–351)	288 (172–366)	263 (205–386)	312 (189–430)
	Open	256 (188–381)	211 (183–287)	242 (183–305)	332 (266–369)	350 (277–431)
VCAM-1 ng/ml (<1,500)*	Laparoscopic	1,062 (828–1,361)	1,049 (760–1,545)	1,070 (838–1,778)	1,696 (1,409–3,018)	1,719 (1,191–1,960)
	Open	986 (738–1,642)	801 (695–1,953)	997 (678–1,882)	1,741 (1,171–3,083)	1,713 (1,051–2,465)

Inflammatory markers in bold indicate significant difference in plasma concentration between treatment groups ($p < 0.05$)

Figures in bold indicate significant pairwise comparison mean differences between types of surgery at individual timepoints

* Normal value in healthy subjects

§ No measurements taken at this timepoint

host is increasingly immuno-suppressed as the disease progresses. The immune response is shifted from a Th1 to a Th2 type response (Evans et al. 2006). The shift from a cell-mediated response to a humoral response increases circulating levels of cytokines such as IL-4, IL-6 and IL-10. Increased levels of IL-10 have been associated with less responsiveness to treatment and a shorter survival for patients with colorectal cancer. Whether the extent of the surgical trauma and its immunological consequences really has an impact on the cancer disease is not fully known.

Our study also showed a significantly lower level of CRP in the laparoscopic group. This finding has also been shown in other studies. CRP is an acute phase protein and is elevated after trauma and infection. In a study by Aleksandrova et al. (2010), the result suggested that elevated CRP might be a risk-factor for development of colon but not rectal cancer. Chronic inflammation might be of importance in the development of cancer. Recent evidence suggests that anti-inflammatory treatment with aspirin reduces the risk for development of colorectal cancer. The required doses are higher than recommended for cardiovascular prevention (Asano and McLeod 2004; Chan et al. 2005; Flossmann and Rothwell 2007).

The duration of surgery was significantly longer in the laparoscopic group. The fact that laparoscopy is less invasive and causes less tissue trauma seems to be more important than the duration of surgery. The peri-operative bleeding was significantly higher in the open group. Bleeding is also known to cause an inflammatory response. There are possible explanations for less bleeding in laparoscopic surgery as compared to open. The positive pressure of pneumoperitoneum stops smaller bleeding. This study was not powered to investigate differences in clinical parameters between surgical groups. It would be useful to investigate further if the differences in inflammatory response also mirror a difference in post-operative complications such as SIRS, sepsis and MODS.

In conclusion, rectal surgery causes a release of CRP, pro- and anti-inflammatory cytokines in the peri-operative and in the early post-operative phase. The pro- and anti-inflammatory response to rectal surgery was less pronounced in laparoscopic rectal surgery as compared to open. Less tissue trauma in laparoscopic rectal surgery and/or less peri-operative bleeding in the laparoscopic cases lead to a lower degree of inflammatory response.

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Conflict of Interest Drs Kvarnström, Swartling, Kurlberg, Bengtson and Bengtsson have no conflicts of interest or financial ties to disclose.

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