METABOLIC AND ONCOLOGICAL CONSEQUENCES OF LAPAROSCOPIC SURGERY

METABOLE EN ONCOLOGISCHE GEVOLGEN VAN LAPAROSCOPISCHE CHIRURGIE

PROEFSCHRIFT

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To Marcel

and my parents

Contents

1

Brief History of Laparoscopy

Introduction

In 1986, Philip Mouret and his colleagues performed the first laparoscopic cholecystectomy[1]. They initiated the most revolutionary change in traditional surgery, since the introduction of anaesthesia, asepsis, antibiotics and blood-transfusion.
At the same time, industry propelled this development by constantly introducing superior and more sophisticated equipment, as well as by organizing training-courses.
The use of pre-existing and/or non-existing openings in the human body for both diagnostic examination and therapy has always been a matter of technological feasibilities. Since endoscopic surgery has rapidly become a popular alternative of traditional surgery for a high number of interventions, numerous historical papers and commentaries have been published, in which many claimed to have been the first[2,3].

Optics and light source in endoscopy

As early as in the 10th Century, an Arabic physician, Abulkasim (936 - 1013) examined the cervix by means of reflected light, followed by others who observed nose and bladder[4]. In 1806 Bozzini (Frankfurt) wrote an article on the 'Lichtleiter' he had invented, consisting of a double-lumen cannula, a concave mirror and a candle, which he used to examine a woman's bladder. With this invention, he had demonstrated that it was possible to look into natural human body-openings[5]. Desormeaux substituted the candle for a paraffin lamp (1843). Bruck, a dentist, introduced electricity, using a platinum incandescent filament. Until that moment the device of scopic vision consisted of a light source and a hollow tube only. In 1879, a German named Nitze added an optical lens to the tube[6]. After the introduction of the light bulb by Edison, the candle and paraffin lamp were no longer used as a light source. In first instance a miniature light was installed on the scope itself, causing specific risks due to the high

temperature of the lamp. Therefore, this mechanism was preferably used to examine the bladder, making use of urine for cooling.

In 1929, the German hepatologist Kalk described the advantages of a 135° lense-system, enabling examination "around the corner"[7]. Although fiberoptics were patented as early as in 1928, this new technology was applied only in 1952, transporting light from a powerful light-source outside the body to the inner parts via a scope. This so-called 'cold light' was introduced in flexible scopes from 1957 onwards. Several developments further improved the use of the scope. In 1966 Hopkins, a British physician, installed quartz-lenses on short distances in scopes. In addition, by attaching a coating layer of magnesium-fluoride on the surface of the lenses the loss of light caused by reflection was reduced substantially[8]. The halogen light source and more recently, the introduction of Xenon-light have further improved the scope-mechanism.

Laparoscopy

As soon as a suitable scope was available to inspect the inner body through natural entries, attempts were undertaken to use this equipment to examine the abdomen and thorax. In 1901 von Ott and Kelling were the first to inspect the abdominal cavity. Dimitri von Ott, a gynaecologist from Petrograd, placed a scope in the pelvis through an incision of the fornix anterior, after positioning a patient in 45° Trendelenburg. In this position, he observed the position of the bowels against the diaphragm and named this technique *ventroscopy*[9]. Kelling conducted the same operation on a dog. The abdomen was air-insufflated by using a Nitze cystoscope through a trocar. Kelling (Dresden) called this *celioscopy*. These procedures were purely diagnostic[10]. In 1910, Jacobaeus (Stockholm) applied the technology without pneumoperitoneum for evaluation of patients suffering from ascites and liver-diseases. Afterwards the technology was also used in the thorax for the treatment of tuberculosis, bringing Jacobaeus fame for being the founder of thoracoscopy[11]. Bernheim, general surgeon at the John Hopkins University Hospital, was the first to perform laparoscopy using a ½

inch proctoscope and an electric head-lamp (1911). He named this procedure *organoscopy*. Between 1929 and 1959, Kalk wrote 21 articles on laparoscopy, including an article in 1951 on a personal series of 2000 without mortality. Kalk also introduced the method of using two trocars[12].

Pneumoperitoneum

In addition to light source, optics and scope the development of the pneumoperitoneum has been of prime importance for the progress of laparoscopy. With the exception of Kelling in 1901, the pneumoperitoneum was not used until 1918, when Goetze (1918) and later Veress (1938) developed a spring-loaded obturator that was incorporated within the barrel of the needle, enabling simultaneous abdominal punction and gas-insufflation[10,13,14]. The risk of a bowel lesion or damage to vessels was substantially reduced. Initially this device was used for creation of an artificial pneumothorax for the treatment of tuberculosis. The shape of this needle has not changed ever since. In 1946, Decker introduced an alternative method of placing the laparoscope into the abdominal cavity in an attempt to minimize the risk of complications. The patient was placed in a knee-chest position so that a pneumoperitoneum developed spontaneously. He inserted the scope into the pelvis through the cavum Douglasi (de 'cul-de-sac') and named this procedure *culdoscopy*[15]. The technique of performing infraumbilical puncture after lifting the abdominal wall was not described until 1959 when Menken reported no complications in a series of 3000 cases. In 1920 it was Ordnoff, an American, who invented the trocar with a pyramidal point and a valve to prevent the escape of air[16]. As laparoscopic procedures became more widely accepted, increasing numbers of complications were seen (1% complication rate). This situation prompted Hasson in 1974 to introduce a cannula for open laparoscopy, which helped to decrease the number of vascular and bowel injuries[17].

The gas used to insufflate the abdominal cavity varied from filtered open air to pure

oxygen. In 1924, it was Zollikorfer (Switzerland) who advised to use carbondioxide as the preferred insufflating gas, because of its easy and fast resorption[18]. In 1947 Palmer recommended a maximum intra-abdominal pressure of 25 mmHg. Only in 1977 Kurt Semm (Kiel) introduced the CO_2 Pneu-Automatic Insufflator, facilitating automatic monitoring of the intra-abdominal pressure, which led to the discontinuation of manually insufflating the abdomen[19].

Development of laparoscopy in surgical practice

Initially, especially gynaecologists promoted the use of the laparoscope. Already in 1933 Fervers, proclaimed adhaesiolysis and biopsies to be performed via this method[20]. Anderson performed the first laparoscopic sterilisation in 1937, whilst in the same year Hope evaluated the first ectopic pregnancy.

Semm played a vital role in the development of laparoscopic instruments and laparoscopic techniques. Eventually, he performed approximately 75 percent of his gynaecologic procedures laparoscopically, with a low complication rate (0.28 %)[19]. He has, therefore, clearly demonstrated that laparoscopic surgery is safe and less traumatic than conventional surgery. Nevertheless the first publications on laparoscopic appendectomy (Kok, 1977 and Semm, 1983) were strongly criticized[21,22]. Initially laparoscopic visualization of the abdominal cavity was restricted to the individual directing the operative procedure. Participation by other surgeons was therefore limited. In 1986, however, this problem was solved by the development of a computer chip TV camera attached to the laparoscope. Video imaging facilitated education and instruction of surgeons.

Laparoscopic cholecystectomy heralded the new era of surgery. By 1990 Dubois had already conducted 36 laparoscopic gallbladder operations[23]. Other laparoscopic techniques followed rapidly, such as appendectomy, herniorrhaphy, colon surgery, splenectomy, nephrectomy, gastrectomy, adrenalectomy, vagotomie, laparoscopic

antireflux surgery and even pancreatectomy. Whilst a decennium before, all of these areas were still underdeveloped and approached with suspicion by general surgeons, new laparoscopic procedures are now accepted almost unconditionally.

This thesis is an attempt for an improved and deeper understanding of the role of laparoscopy in malignant colon surgery. Since experiments were conducted in rats, special laparoscopic instruments were used, that have only recently been developed.

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2

General Introduction and Objectives

Introduction of laparoscopic techniques in general surgical practice started in the late 1980's following the initial reports describing the technique of laparoscopic cholecystectomy[1,2]. Presently, the laparoscopic approach is the operation of choice in patients with gallbladder disease, recurrent inguinal hernia, gastro-esophageal reflux and small adrenal tumors[3]. The laparoscopic approach involves exposure of the abdominal cavity to gases or mechanical devices and the use of minimal access sites, which allow introduction of laparoscope and instruments. The use of minimally invasive surgery appears to be associated with reduced postoperative pain, shorter hospital stay, less operative trauma, decreased systemic stress response and earlier return to normal activity than conventional surgery[4,5,6]. In 1991 laparoscopic colon surgery was first published[7]. Following reports have shown that laparoscopic colorectal surgery is technically feasible, safe, and capable of fulfilling oncologic criteria for cancer surgery[8,9]. Laparoscopic resection of colorectal tumors uses the same principles of excisional surgery of malignant colorectal tumors[10,11]. However, several factors have impeded widespread application of laparoscopic colorectal surgery. Advanced laparoscopic skills, costly and technically complex disposable instruments and long occupation of the operation room are predicaments of laparoscopic colorectal surgery. In addition, concern about the extent of laparoscopic resection of lymphatic tissue and reports of port site metastases have held back the majority of surgeons to remove colorectal cancers laparoscopically[12,13,14]. Over twenty publications have reported more than 30 tumor recurrences at the site of cannula insertion or at the site of extraction of the specimen after laparoscopic colorectal surgery[15,16]. Vukasin et al reported in the largest series until now that the incidence of wound recurrence after 480 laparoscopic colectomies was 1.1%[17]. Tumor recurrence in the abdominal wall after open surgery has been rarely reported. In a series of 1603 conventional resections of colorectal cancer, 11 patients developed abdominal wall metastases in the laparotomy incision or at the insertion site of a drainage tube, accounting for a rate of 0.6%[18]. Similarly,

Reilly et al reviewed 1711 patients with colorectal cancer who were treated curatively and found that 26 patients had abdominal wall or perineal recurrence, accounting for an incidence of 1.5%. Incisional involvement could only be documented in 11 (0.6%) of these cases[19]. Although the true incidence of abdominal wall metastases after open resection of colorectal cancer is unknown, abdominal wall metastases after laparoscopic resections of colorectal cancer seem to be more prevalent. Randomized studies with adequate follow-up are necessary to establish differences in abdominal wall recurrence rates and prognosis after either laparoscopic or conventional surgery.

Prior to clinical employment of laparoscopic techniques to remove malignant tumours, experimental studies are necessary to determine the effects of laparoscopy on tumor biology and physiology. Several experimental studies have shown that surgery may facilitate tumour growth[20]. In these studies, the extent of the operative trauma appeared to be related to the degree of tumour growth stimulation[21]. In addition the results of a recently performed clinical trial on the effect of blood transfusions suggested a similar relationship between the extent of surgical trauma and prognosis. In this trial, regardless of the type of blood transfusion, transfusions were associated with poor prognosis, probably because of the circumstances that necessitated them i.e. surgery[22]. The operative trauma associated with laparoscopic surgery is considered to be less than that of conventional surgery[23]. The extent of postoperative metabolic and immunologic changes is proportional to the degree of surgical trauma[24,25]. Clinical studies comparing laparoscopic and conventional surgery show higher glucose, epinephrine and norepinephrine responses after conventional surgery[26]. These observations suggest that laparoscopic surgery is associated with less metabolic stress than open surgery. Other clinical studies on immune response found lower levels of interleukin-6 (IL-6) and preservation of cell-mediated immune function after laparoscopic surgery[5,27]. Recent studies suggest that IL-6 is a marker of injury severity and a predictor of postoperative complications[28]. Considering these facts, our hypothesis is that laparoscopic surgery could be associated with metabolic and oncological benefits over conventional surgery.

To assess the role of laparoscopic techniques to treat malignant disease, we developed an experimental tumor model which mimicked the clinical situation of patients with colorectal cancer. Until now, large animals have been the preferred model for laparoscopic training and research[29]. However, these animals are expensive, and detailed knowledge of some aspects of their physiology is limited. Therefore, in this thesis a laparoscopic rat model was used which allows detailed investigation of certain physiologic and immunologic consequences of laparoscopic surgery[30]. The optimal tumor model would be a localized tumor that could be removed laparoscopically. Such a tumor model is still not available. In addition, other models were used to imitate different aspects of tumor behaviour during and after laparoscopic and conventional surgery. Subsequently, metabolism and tumor biology related to different types of laparoscopic surgery, gasless, air and CO_2 pneumoperitoneum were studied in a rat model.

In the *cell-seeding model,* tumor cells were injected intraperitoneally directly after surgery (tumor take) and two days before each procedure (tumor growth). Tumor take was measured to search for differences in grafting after different types of surgery. Tumor growth was measured to search for differences in growth of established intraperitoneal tumor cells after different types of surgery. Furthermore intraperitoneal free tumor cells, injected intraperitoneally prior to surgery were used to mimic the clinical situation of free viable tumor cells and to investigate the mechanisms of abdominal wall recurrence.

In the *solid tumor model,* a lump of tumor cells was placed intraperitoneally after different types of surgery and thereafter removed through a laparoscopic port or through the midline laparotomy. This model was developed to improve understanding of the tumor growth in scars after extraction. The *subrenal capsule assay* (SRC) allows objective determination of tumor growth because the subcapsular tumor can be dissected easily and weighed accurately[31]. This assay was used to study systemic

changes caused by different types of surgery which have influence on extraperitoneal tumor growth. Furthermore we developed a model to study the role of *local ischemia* and *gas leakage* on the development of abdominal wall metastases.

To study differences in metabolism after laparoscopic surgery and conventional surgery postoperative *bodyweight* was measured. Bodyweight is supposed to reflect anabolic state. Since bodyweight is a rough measurement of metabolic state, we tried to find an anabolic hormone with a relatively long half life time so that measurements during the day would not fluctuate greatly. *Insulin-like growth factor 1* (IGF-1) appears a representative parameter of anabolism. The insulin-like growth factor family of peptides, binding proteins, and receptors are important for normal human growth and development, and are involved in specialized functions of most physiologic systems[32,33]. Besides, different cancer cells express members of the IGF system. Consequently the IGF system may play an important role in the reproduction of malignant cells. New therapies which modulate various components of the IGF system could affect the progression and metastasis of cancer[34,35].

The objectives of this thesis can be summarised as follows:

1. to analyse the oncological benefits of laparoscopic surgery in comparison to conventional surgery, with specific emphasis on
   - intraperitoneal tumor take
   - intraperitoneal tumor growth
   - tumor growth in the subrenal capsule assay

2. to investigate the metabolic aspects of laparoscopic surgery as opposed to conventional surgery, specifically
   - body weight
   - insulin-like growth factor 1 levels

3. to study the influence of different aspects of laparoscopic surgery on the development of abdominal wall metastases, including
   - direct tumor-wound contact
   - different insufflation gases
   - gasless laparoscopy
   - gas leakage
   - ischemia

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Laparoscopic Surgery in the Rat: Beneficial Effect on Body Weight and Tumor Take

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Surgical Endoscopy 1996;10:490-494.

Abstract

Background: The ability of laparoscopic techniques to treat malignant disease is controversial. We developed a rat model to assess metabolic and oncological effects of laparoscopic surgery.

Methods: Experiment I. The postoperative body weight in 10 rats having laparoscopic bowel resection (group I) and 10 rats having open bowel resection (group II) and 5 rats having anesthesia only (group III) was determined. Experiment II. Tumor take was scored in 11 rats having laparoscopic bowel resection (group IV), 11 rats having open bowel resection (group V), 6 rats having CO_2 pneumoperitoneum without bowel resection (group VI) and 6 rats having anesthesia only (group VII). All rats had CC531 cancer cells injected intraperitoneally postoperatively.

Results: Experiment I. Body weight loss in group I compared to group II ($p=0.036$). Rats of group III lost no weight postoperatively. Experiment II. Tumor take was less in the subcutis ($p=0.005$), parietal peritoneum ($p<0.001$) and bowel anastomosis ($p=0.021$) in group IV compared to group V. Tumor take was significantly greater at all sites except for subcutis in group VI compared to group VII (all $p<0.022$).

Conclusions: Laparoscopic surgery is associated with less postoperative weight loss and less tumor take compared to open surgery. CO_2 insufflation appears to increase tumor take.

Key words: Laparoscopy - Rat - Tumor take - Weight loss - Pneumoperitoenum - Bowel resection

Introduction

The revolutionary introduction of laparoscopic techniques to treat gallbladder disease has stimulated clinical investigators to use this technology for other procedures such as appendectomy, hernia repair, and Nissen fundoplication. Most laparoscopic techniques are associated with decreased post-operative pain, less post-operative disability, shorter hospital stay and earlier return to normal activities in comparison to conventional surgery[8,15,20].

Application of laparoscopic techniques in colorectal surgery seems to be a logical next step in the evolution of laparoscopic surgery. The first report of laparoscopic colectomy in 20 patients was published in 1991, and seemed promising[12]. However, the total number of patients having laparoscopic colorectal resections is small in comparison to the wide extent of application of laparoscopic cholecystectomy. The technical complexity, long duration and the high financial costs of laparoscopic colorectal procedures are probably responsible for the slow expansion of this type of surgery[2]. However, the most important impeding factor seems the general concern about the feasibility of laparoscopic techniques to treat malignant disease. In addition to concern about the extent of laparoscopic resection of lymphatic tissue, reports on port site metastases after laparoscopic resections of colon malignancies have startled colorectal surgeons. A case report in 1993 of a patient with abdominal wall recurrence after laparoscopic right hemicolectomy was followed by several other publications[6,21].

To assess the role of laparoscopic techniques in the treatment of malignant disease, experimental studies are necessary. At the present, large animals are the preferred model for laparoscopic training and research[4]. However, these animals are expensive, and detailed knowledge of some aspects of their physiology is limited. Therefore, in this study a laparoscopic rat model was used to investigate the physiologic and immunologic consequences of laparoscopic surgery[3]. In addition, the use of cultured tumor cell lines allows study of the effects of laparoscopic and open surgery on tumor

take in such an experimental model.

This article reports two experiments assessing the postoperative changes of body weight and differences in tumor take after laparoscopic or open small bowel resections in rats.

Materials and Methods

Animals

Male rats of the inbred WAG strain, weighing 200-300 g and aged 4-5 months were obtained from Harlan-CPB, Austerlitz, The Netherlands. Rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (Temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark) and were fed a standard laboratory diet (Hope Farms, Woerden, the Netherlands) with free access to water and food before and after surgery. The protocols were approved by the Committee on Animal Research of the Erasmus University, Rotterdam.

Tumor

CC-531 is a 1,2-dimethylhydrazine-induced, weakly immunogenic, moderately differentiated colon adenocarcinoma, transplantable in syngeneic WAG rats. The tumor was maintained in vitro in RPMI 1640 medium supplemented with 5 % fetal calf serum (virus and mycoplasma screened), 1% penicillin (5000 U/ml), 1% streptomycin (5000 U/ml) and 1% L-glutamine (200mM). All supplements were obtained from Gibco (UK). Before their use, cells were trypsinized (5 minutes,37°C), centrifuged (5 minutes, 700xg), resuspended in RPMI 1640 and counted. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). Viability always exceded 95%. The CC-531 tumor is immunogenic as determined by the immunization-challenge method of Prehn and Main[13].

Operative Procedures

Four different operative procedures were performed: anesthesia only, CO_2 pneumoperitoneum only, laparoscopic small bowel resection and resection of the small bowel through a laparotomy.

Anesthesia was achieved with 0.2 mg/ml fentanyl and 10 mg/ml fluanisone (0.5 ml/kg i.m.). The animal was secured to the operating table with adhesive tape in a supine position. The abdomen was shaved and cleaned with 70% alcohol and dried with gauze. The laparoscope, camera and attached cables were held at the desired angle by a flexible arm. The surgeon was sitting at one end of the operating table facing the video monitor. The laparoscopic equipment and instruments (shortened to a length of 130 mm and with a diameter of 2 mm) used, were provided by Karl Storz Endoscopes and are listed in table 1. The instruments and laparoscope were cleaned with alcohol 70% before and after surgery.

The rats having a laparoscopic small bowel resection had a 5-mm skin incision in the midline of the abdomen at two-third between the xiphoid process and the pubis. A 5-mm laparoscopic sheath with insufflation side port was introduced followed by introduction of a 4-mm arthroscope. The pneumoperitoneum was created with CO_2 to a maximum pressure of 4 mm Hg. One operating port was placed in the right, and another in the left upper quadrant of the abdomen under direct vision. The operating ports consisted of a shortened venous catheter with side port/homeostasis valve catheter (Arrow, Reading, Pennsylvania). The use of this operating port facilitated the insertion and withdrawal of instruments. The valve prevented CO_2 leakage through the port. After extracting a segment ileum of 8 cm in length, a small bowel resection (length 4 cm of the ileum) was performed laparoscopically. The anastomosis was performed extra-corporeally with a running suture using 7-0 Perma-hand Seide, Ethicon. The ports in the abdomen were closed with one suture, in one layer including muscle and skin (2-0 NC-Silk, B.Braun Melsungen AG).

Rats having only a CO_2 pneumoperitoneum group had a CO_2 pneumoperitoneum for twenty minutes with a maximum pressure of 4 mm Hg.

In the open small bowel resection group, a 5 cm abdominal skin incision was made in the midline of the abdomen. During operation, the viscera were removed from the abdominal cavity, put in phosphate buffered solution (PBS) wettened gauze and left extra-abdominally for approximately 15 minutes during which time a small bowel resection of 4 cm length took place. The anastomosis was made as mentioned above, the viscera were returned to the abdominal cavity and the abdomen was closed in one layer.

The operative time varied between 30 and 45 minutes in all procedures.

Equipment
Small animal laparoscopy table
Insufflator and CO_2 tank*
Cold light projector*
CCD videocamera*
Triniton colour video monitor**
Instruments
4-mm 0° arthroscope*
5-mm metal arthroscope sheath*
2-mm laparoscopic needleholder*
2-mm laparoscopic tissue grasper*
2-mm laparoscopic scissors*

Table 1. Laparoscopic equipment and instruments used for surgery in the rat.
*Karl Storz Endoscopes, **Sony.

Experimental groups

Experiment I: Ten rats (group I) underwent a laparoscopic small bowel resection and were compared with ten rats (group II) which had an open small bowel resection. Group III consisted of five rats which had anesthesia only. The pre-operative weight and post-operative weight of the rats were determined daily for seven days. The weight loss was determined as the percentage weight loss of the pre-operative weight.

Experiment II: Eleven rats (group IV) underwent a laparoscopic small bowel resection

and were compared with eleven rats (group V) which were subjected to an open small bowel resection. Group VI (n=6) had a CO_2 pneumoperitoneum for a period of twenty minutes without small bowel resection. Group VII (n=6) had anesthesia only for a period of 35 minutes. Directly after both types of surgery (group IV and V), anesthesia (group VI) or CO_2 pneumoperitoneum (group VII), $5x10^5$ CC-531 tumor cells in 1.0 ml RPMI 1640 medium were injected intraperitoneally along the inner left and right abdominal walls, respectively. To ensure that the cells were were injected intraperitoneally, the drop test was performed. In this test, a drop of saline solution is placed within the open lumen (the hub) of the tumor cell injection needle. As the needle enters the peritoneal cavity, the relative negative intra-abdominal pressure pulls the fluid through the needle.

After 4 weeks, the animals were sacrificed and tumor-take was scored semi-quantitatively on the following sites: subcutaneously (at the site of the operative scar), parietal peritoneum (operative scar(s)), bowel anastomosis, kidney, liver, retroperitoneum, omentum, and scrotal fat. The scoring ranged from 0-5 and was performed by two independent observers. In case of disagreement between the two observers, the score was middled. A score of 0 indicated no presence of tumor, a score of 1 correlated with an estimated tumor diameter less than 0.5 cm, a score of 2 with a tumor diameter between 0.5 cm and 1 cm, a score of 3 with a tumor diameter between 1 cm and 2 cm, a score of 4 with a tumor diameter between 2 cm and 3 cm, and a score of 5 with a diameter exceeding 3 cm.

Statistics

The mean and the standard deviation (SD) of the collected data were recorded. To analyze the variable data, the Mann-Whitney test was used to assess the presence of a normal distribution of the data. In case of a normal distribution, the unpaired two-tailed Student's *t*-test was used to calculate *P* values. The Fisher Exact test was used to compare binominal observations.

Results

Experiment I : Post-operative weight of the rats

The mean loss of body weight (percentage of pre-operative body weight) of the rats differed significantly between group I and II 2 days post-operatively (p=0.036) and 3 days post-operatively (p=0.002) (figure 1.) After 6 days both groups regained weight. The mean total postoperative weight loss was then 2.36 % ± 1.69 % in group I versus 6.63 % ± 3.49 % in group II (p=0.003). Laparoscopically treated rats started to gain weight after two days and the open treated rats started to gain weight after three days (Fig 1). Rats of group III, which had anesthesia only, retained their normal weight for 2 days and thereafter gained 0.8 % ± 0.3% of their preanesthetic body weight, daily for 5 days (Fig. 1).

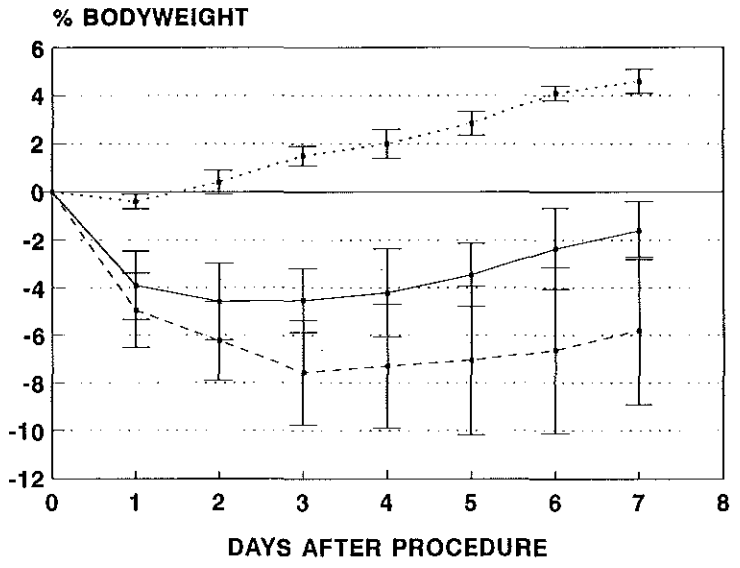

Figure 1. Postoperative loss of bodyweight (expressed as a percentage of the preoperative bodyweight) in rats having laparoscopic small bowel surgery (···■···), rats having open small bowel resections (----■----) and rats having anesthesia only (···■···).

Experiment II: Post-operative tumor-take

Significant differences in tumor take in the subcutis (p=0.005), parietal peritoneum (p<0.001) and bowel anastomosis (p=0.021) were found between the rats having laparoscopic an open small bowel resections (Table 2). Differences in tumor take at the other 4 sites were not significant using the unpaired two-tailed Student's *t*-test (Table 2). The mean tumor take on the kidney was 0.64 ± 0.5 in group IV (laparoscopic) versus 1.00 ± 0.0 in group V (open) (p=0.090). To analyze these binominal data the Fisher Exact test was used for this observation.

Abdominal Sites	Laparoscopic small bowel *(n=11)*	Open small bowel *(n=11)*	*p-value*
Subcutis	0.55 ± 0.93	2.18 ± 1.47	0.005
Peritoneum	1.18 ± 0.75	3.36 ± 1.36	< 0.001
Anastomosis	2.27 ± 1.01	3.64 ± 1.50	0.021
Liver	0.73 ± 0.90	1.45 ± 0.93	NS
Retroperitoneum	0.73 ± 0.65	1.27 ± 0.90	NS
Omentum	1.45 ± 0.69	1.73 ± 0.90	NS
Scrotal fat	1.36 ± 1.03	1.64 ± 0.92	NS
Kidney	0.64 ± 0.5	1.00 ± 0.0	NS

Table 2. Mean tumor take and standard deviation in rats having laparoscopic small bowel resection (group IV) and rats having open small bowel resection (group V).

Group VI, which had had a CO_2 pneumoperitoneum for twenty minutes without small bowel resection showed no differences of tumor take as compared to the rats which had laparoscopic small bowel resections but significant differences in tumor take compared to the rats having open small bowel resections (Figs 2 and 3).

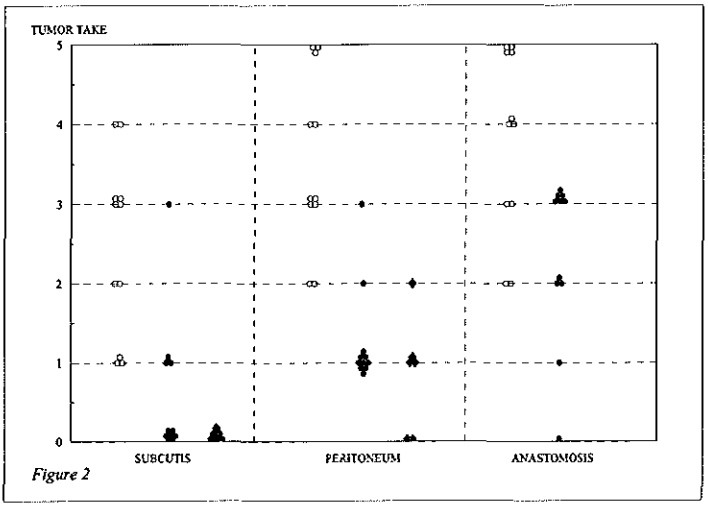

Figure 2. Graphic presentation of statistically significant different tumor take in rats having open small bowel resection (o), rats having laparoscopic small bowel resection (•), and rats having anesthesia only (♦).

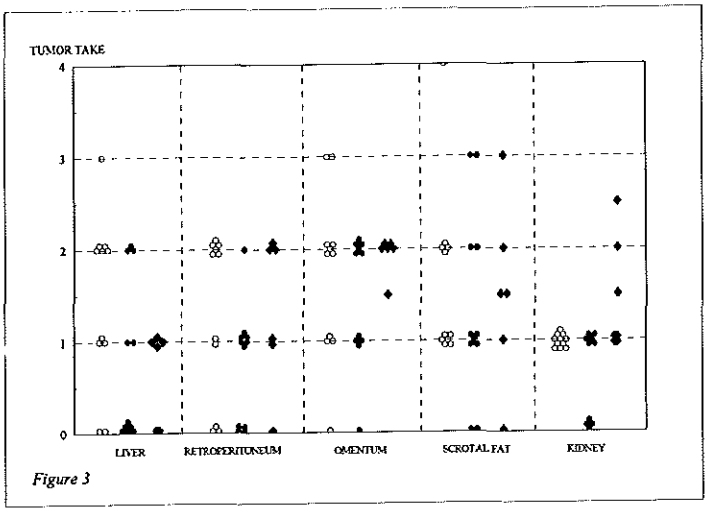

Figure 3. Graphic presentation of statistically non-significant different tumor take in rats having open small bowel resection (o), rats having laparoscopic small bowel resection (•), and rats having anesthesia only (♦).

Group VII, which had anesthesia only without small bowel resection or CO2 pneumoperitoneum showed no tumor take at any of different intraabdominal sites. Differences in tumor take between the CO_2-only group and the anesthesia only group are shown in Table 3.

Abdominal Sites	Pneumoperitoneum (n=6)	Anesthesia only (n=6)	p-value
Subcutis	0 ± 0	0 ± 0	NS
Peritoneum	0.83 ± 0.75	0 ± 0	0.022
Liver	0.67 ± 0.52	0 ± 0	0.010
Retroperitoneum	1.33 ± 0.82	0 ± 0	0.003
Omentum	1.92 ± 0.20	0 ± 0	<0.001
Scrotal fat	1.50 ± 1.00	0 ± 0	0.004
Kidney	1.42 ± 0.49	0 ± 0	<0.001

Table 3. Mean tumor take and standard deviation in rats having CO_2 pneumoperitoneum (group VI) and rats having anesthesia only (group VII).

Discussion

Laparoscopic assisted colorectal surgery has been shown to be technically feasible and associated with an acceptable low level of morbidity[2,12,20]. However, there is little known about long term survival and the incidence of abdominal wall recurrence after laparoscopic resection of colorectal malignancies[6,21]. A prospective randomized trial is mandatory to establish the role of laparoscopic techniques in the treatment of colorectal cancer. Ethical dilemmas and interindividual variability of patients have compromized such studies in human. Therefore, laparoscopic studies in experimental animals are indicated to assess the value of laparoscopic surgery in treating malignancies of the colon[1].

In this study, a laparoscopic rat model was used which proved to be simple, effective and low in cost[3]. This experimental model appears to correlate well with the clinical situation because reconvalescence, reflected by post-operative weight, after laparoscopic surgery is significantly improved in comparison to open surgery. Therefore, we consider the laparoscopic rat model appropriate to investigate the consequences of laparoscopic surgery. The purpose of this study was to determine if the known metabolic advantages of laparoscopic surgery are parallelled by advantages regarding tumor clearance. This study has shown that the tumor take is significantly less after laparoscopic surgery. This finding is in accordance with the report by Allendorf et al. who also found significantly less subcutaneous tumor take after laparoscopic surgery[1]. The mechanism of decreased tumor take after laparoscopic surgery remains to be elucidated. A possible factor could be the use of minimal accesses in laparoscopic surgery resulting in decreased operative trauma. Lesser operative trauma is known to be associated with decreased attenuation of the immune defense mechanisms[7,19]. Other evidence of improved immunologic preservation after laparoscopic surgery is provided by Trockel who reported that delayed-type hypersensitivity is better preserved after laparoscopy than laparotomy in a rat model[16]. In addition to experimental studies, clinical studies have demonstrated higher levels of both serum and peritoneal fluid interleukin-6 after open cholecystectomies in comparison to laparoscopic cholecystectomies[14,17]. Interleukin-6 is a cytokin which augments inflammation, stimulates B-cells to grow and to produce polyclonal immunoglobulins.

In spite of the reduced tumor take after laparoscopic small bowel resection, the finding of a significant difference in tumor take between rats having only CO_2 pneumoperitoneum and rats having only anesthesia without CO_2 pneumoperitoneum is concerning. This observation suggests a negative role of CO_2 on the development of tumor implants. Recently, CO_2 was reported to induce decreased peritoneal tissue macrophage activity as compared to laparoscopy with air inflation and laparotomy[18]. The role of CO_2 in the pathogenesis of abdominal wall metastases is under debate[5,9]. Carbondioxide could act as a vehiculum of tumour cells transporting cells from the

tumor to sites susceptible to tumor take due to ischemia (trocar wounds) or tissue trauma (intestinal anastomosis or abdominal wounds)[10,22]. These considerations mandate evaluation of gasless laparoscopy in experimental and clinical setting.

In conclusion, in this study, laparoscopic surgery is associated with reduced tumor take. Use of carbondioxide appears to stimulate tumor take. Further studies are required to assess the definitive role of laparoscopic surgery in the treatment of malignant disease.

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Letter to the Editor

Dear Sir,

We read with interest the paper from Drs Bouvy et al, who have provided some helpful insights into the behaviour of malignant tumour cells during laparoscopic surgery[1]. As with work previously reported by Allendorf et al, they have demonstrated that laparoscopic procedures may be associated with reduced growth of tumour cells in a small animal model[2]. This may be due to reduced physiological stress in animals undergoing laparoscopic surgery, resulting in less perioperative suppression of the immune system.

However, we would caution readers not to draw the conclusion that laparoscopic approaches the resection of abdominal malignancy are safe. In Dr Bouvy's study, cancer cells were not introduced into the peritoneal cavity until after the surgical procedure was concluded. Therefore the potential for cancer cells to be redistributed by insufflation of CO_2 gas and cause of wound metastases was not tested.

Our own recent experience with laparoscopic and open surgical laceration of an implanted adenocarcinoma in a rat model has demonstrated a five-fold increase in the incidence of metastases in the abdominal access wounds of rats undergoing laparoscopic surgery, despite the advantage of reduced growth of the implanted 'primary' tumour[3]. Similar results have been reported by Jones et al following introduction of free tumour cells into the abdominal cavity at the commencement of either laparoscopy with CO_2 insufflation or laparotomy in a hamster model[4]. These studies suggest that if cancer cells are liberated during prolonged CO_2 insufflation, then metastatic implantation and growth in the abdominal wall wounds is more common

following laparoscopic surgery. Interestingly, a further experiment using the same implanted tumour model in our institution during laparoscopic tumour laceration at both gasless and conventional laparoscopy, demonstrated a threefold reduction in the incidence of wound metastases following gasless laparoscopy (unpublished data).

It would be interesting to repeat the experiments performed by Drs Bouvy et al, with the tumour cells introduced into the peritoneal cavity at the commencement. rather than the conclusion of the laparoscopic and open surgical procedures. The effect of pneumoperitoneum would then be directly tested and the pattern of tumour implantation may be significantly altered. Until the issue of laparoscopic dissemination of malignant cells is fully understood, patients should not undergo laparoscopic surgery for malignancy outside the context of controlled clinical trials.

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Authors reply

We thank Drs. Watson and Mathew for their valuable commentary on our paper: "Laparoscopic surgery in the rat: Beneficial effect on body weight and tumor take"[1]. Our hypothesis is that the reduced surgical trauma of laparoscopy is associated with less tumor take and growth. Experimental studies in rats have confirmed that tumor take and growth after laparoscopic surgery are significantly less than after laparotomy[1,2,3].

However, tumor growth and take in these studies were considered to represent the general response to surgical trauma. In our opinion, port site metastases are caused rather by local than general factors. Direct implantation of tumor cells in the abdominal wall during extraction of a specimen and displacement of free viable intraperitoneal tumor cells have been suggested as important factors in the development of abdominal wall metastases. Insufflation of gas in the peritoneal cavity appears to provide an excellent vehiculum to transport the free cancer cells to port sites. Leakage of gas through or along trocars, a phenomenon we refer to as the 'chimney effect' probably predisposes to the development of port site metastases[4].

Drs. Watson and Mathew expressed their interest in an experiment with the introduction of tumour cells at commencement. We have recently reported the results of a study on the impact of CO_2 and gasless laparoscopy on abdominal wall metastases in rats[5]. In accordance with the study by Watson and Mathew we found significantly greater port site metastases in rats having CO_2 laparoscopy compared to gasless laparoscopy[6]. Therefore, further study on gasless laparoscopy in digestive cancer is required.

We concur with Drs. Watson and Mathew that the role of laparoscopic surgery in digestive cancer requires further evaluation, both in experimental and clinical studies.

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4

Laparoscopic Surgery is associated with less Tumour Growth Stimulation than Conventional Surgery: an Experimental Study

ND Bouvy, RL Marquet, J Jeekel, HJ Bonjer

British Journal of Surgery 1997;84:358-361.

Abstract

Background: The role of laparoscopic surgery in malignant disease is controversial. To evaluate differences in tumour growth after either conventional or laparoscopic surgery, an experimental study was performed in rats.

Methods: After intaperitoneal injection of CC-531 colon cancer cells or subcapsular renal implantation of CC-531 cancer, rats underwent either laparoscopically assisted small bowel resection, open small bowel resection or anaesthesia only. Peritoneal tumour growth and subcapsular renal tumour growth were assessed after operation.

Results: Peritoneal tumour growth was the least after anaesthesia only ($p<0.05$) and less following laparoscopic resection as compared with open resection ($p<0.05$). Subcapsular renal tumour growth after either laparoscopic resection or anaesthesia only was less than after open resection (both $p<0.01$).

Conclusion: Laparoscopic surgery was associated with less tumour growth than conventional surgery in this experimental study.

Keywords: Laparoscopy - Rat - Tumour growth - Subrenal capsule assay - Colon - Carcinoma.

Introduction

Laparoscopic surgery is generally associated with less post-operative pain, a shorter hospital stay and an earlier return to normal activity than conventional surgery[1,2,3]. As a consequence of the advantages associated with minimally invasive surgery, laparoscopic techniques have also been employed in colorectal surgery. Although almost all colorectal procedures can be performed laparoscopically, laparoscopic colorectal surgery has not developed at the same rate as other laparoscopic procedures. Several factors have impeded widespread application of laparoscopic colorectal surgery. Advanced laparoscopic skills, costly and technically complex disposable instruments and long occupation of the operation room are predicaments of laparoscopic colorectal surgery. In addition, concern about the extent of laparoscopic resection of lymphatic tissue and reports of port site metastases have restrained the majority of surgeons from performing laparoscopic resection for colorectal cancer[4,5,6].

Several experimental studies have shown that operation may facilitate tumour growth[7]. In these studies, the extent of the operative trauma appeared to be related to the degree of tumour growth stimulation[8]. Similarly it has recently been reported that, regardless of their type, blood transfusions are associated with poor prognosis, probably because of the circumstances that necessitate them[9]. The operative trauma associated with laparoscopic surgery is considered to be less than that of conventional surgery[10]. To assess the oncological benefit of laparoscopic over conventional surgery, peritoneal tumour growth and tumour growth under the subrenal capsule was studied after laparoscopic or open small bowel resection in rats.

Materials and Methods

Animals

Male rats of the inbred WAG strain, weighing 200-300 g and aged 4-5 months, were obtained from Harlan-CPB, Austerlitz, The Netherlands. They were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (Temperature 20-24°C, relative humidity 50-60 percent, 12 h dark) and were fed a standard laboratory diet (Hope Farms, Woerden, the Netherlands) with free access to water and food before and after surgery. The protocols were approved by the Committee on Animal Research of the Erasmus University, Rotterdam, The Netherlands.

Tumour

CC-531 is a 1,2-dimethylhydrazine-induced, weakly immunogenic, moderately differentiated colonic adenocarcinoma, transplantable in syngeneic WAG rats. This tumour is weakly immunogenic as determined by the immunization-challenge method of Prehn and Main[11]. The tumour was maintained *in vitro* in RPMI1640 medium supplemented with 5 percent fetal calf serum (virus and mycoplasm screened), 1 percent penicillin 5000 units/ml, 1 percent streptomycin 5000 units/ml and 1 percent L-glutamine 200 mmol/l. All supplements were obtained from Gibco, Paisley, UK. Before use, cells were trypsinized (5 min at 37°C), centrifuged (5 min, 700g), resuspended in RPMI1640 and counted. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). Viability always exceeded 95 percent. All tumour cells were injected within 4 h after obtaining them.

To grow solid tumour, $1x10^8$ CC-531 tumour cells were injected in the right and left flanks of syngeneic WAG rats. After 6 weeks the tumour volume in both flanks reached a volume of 2.5 cm^3 and the tumour mass was isolated aseptically with a

scalpel from the outer membrane of the main lesion. Subsequently the tumour was cut into appoximately 1 mm^3 cubes (weighing 7.6-8.4 mg), immersed in a culture solution, and stored at 4 °C until implantation under the renal capsule. All subrenal implantations were performed within 1-4h after collection of the solid CC-531 tumour from syngeneic WAG rats.

Operative Procedures

After being anesthetized with atropine (Centrafarm, Ettenleur, The Netherlands) 0.05 mg/kg subcutaneously, Domitor (Smithkline Beecham, Zoetermeer, The Netherlands) 0.25 mg/kg intramuscularly and Ketalin (Apharmo, Arnhem, The Netherlands) 40 mg/kg intraperitoneally, the abdomen of the animals was shaved. The rat was secured to the operating table with adhesive tape in a supine position and the abdomen was cleaned with 70 percent alcohol and dried with gauze. The laparoscope, camera and attached cables were held at the desired angle by a flexible arm. The surgeon was sitting at one end of the operating table facing the video monitor. The laparoscopic equipment and instruments (shortened to a length of 130 mm and with a diameter of 2 mm) were provided by Karl Storz Endoscopes (Vianen, The Netherlands) and Duffner (Tuttlingen, Germany). The instruments and laparoscope were cleaned with 70 percent alcohol before and after surgery.

Rats undergoing minimally invasive small bowel resection had a 5-mm skin incision in the midline of the abdomen, two-third of the way between the xiphoid process and the pubis. A 5-mm laparoscopic sheath with insufflation side port was introduced, followed by a 4-mm arthroscope. Pneumoperitoneum was created with CO_2 to a maximum pressure of 6 mmHg. One operating port was placed in the right, and another in the left upper quadrant of the abdomen under direct vision. The operating ports consisted of a shortened venous catheter with side port-homeostasis valve catheter (Arrow, Reading, Pennsylvania, USA). Use of this operating port facilitated the insertion and withdrawal of instruments. The valve prevented CO_2 leakage through the port. After laparoscopic mobilization, a segment ileum of 8-cm in length was extracted

and a small bowel resection (length 4-cm of the ileum) was performed. The anastomosis was performed extra-corporeally with a running suture using 7-0 Perma-hand Seide (Ethicon, Amersfoort, The Netherlans). The ports in the abdomen were closed with one suture, in one layer including muscle and skin (2-0 NC-Silk, B.Braun, Melsungen, Germany). In animals having open small bowel resection, a 5-cm abdominal skin incision was made in the midline of the abdomen. During operation, the viscera were removed from the abdominal cavity, placed in phosphate buffered solution (PBS) wettened gauze and left extra-abdominally for approximately 20 minutes while small bowel resection of 4-cm length took place. The anastomosis was constructed as described above, the viscera were returned to the abdominal cavity and the abdomen was closed in one layer. Operating time varied between 30 and 45 minutes in all procedures. In the anesthesia group, rats were anaesthetized for approximately 40 minutes. To end anaesthesia Antisedan (Smithkline Beecham, Zoetermeer, The Netherlands) 2 mg/kg intramuscularly was used.

Experimental groups

Experiment 1

On day one, $5x10^5$ CC-531 tumour cells in 1.0 ml RPMI1640 medium were injected intraperitoneally along the inner left and right abdominal walls. To ensure that the cells were injected intraperitoneally, the drop test was performed. In this test, a drop of saline solution is placed within the open lumen (the hub) of the tumour cell injection needle. As the needle enters the peritoneal cavity, the relative negative intra-abdominal pressure pulls the fluid through the needle. After two days all rats were randomized between the three groups. Thirteen rats (group 1) underwent an open small bowel resection and were compared with 13 rats (group 2) that had a laparoscopic small bowel resection. Group 3 comprised of eight rats that had anaesthesia only.

After 4 weeks the animals were killed and tumour growth was scored semi-quantitatively on the following sites: subcutaneously (at the site of the operative scar), parietal peritoneum (operative scars), bowel anastomosis, kidney, liver,

retroperitoneum, omentum, and scrotal fat. Scoring ranged from 0 to 5 at each site and was assessed by two independent observers, according to the peritoneal cancer index described by Steller *et al.*[17]. In case of disagreement between the two observers, the score was averaged. A score of 0 indicated no presence of tumour, a score of 1 correlated with an estimated tumour diameter of less than 0.5 cm, a score of 2 correlated with a tumour diameter between 0.5 cm and 1 cm, a score of 3 with a tumour diameter between 1 cm and 2 cm, a score of 4 with a tumour diameter between 2 cm and 3 cm, and a score of 5 with a diameter exceeding 3 cm. The total tumour load of each rat was defined as the total of scores at all abdominal sites.

Experiment 2

On day one, 22 rats had a 2.5-cm midline laparotomy. Both kidneys were exposed and a solid piece of CC-531 colonic tumour weighing approximately 8 mg was placed under the capsule of both kidneys under microscopic vision. During operation, the viscera were covered with (PBS) wetted gauze, as mentioned above. The operative time of this procedure varied between 20 and 25 minutes. Viscera were returned to the abdominal cavity and the abdomen was closed in one layer. Some 2 days later eight rats (group 4) underwent an open small bowel resection and were compared with eight rats (group 5) which were subjected to a laparoscopic small bowel resection. Group 6 (n=6) had anaesthesia only, for a period of 40 min. Some 14 days after tumour implantation all animals were killed and the growth of the subcapsular tumour was measured by weighing of the enucleated lump.

Statistical analysis

The mean and the standard deviation (s.d.) of the collected data were calculated. To analyze the data for significant differences, the analysis of variance (ANOVA) was used. To assess the presence of a normal distribution of the data, a histogram was made. All results proved to be normally distributed. Results were considered statistically significant at $P<0.05$.

Results

Experiment 1. Peritoneal Tumour Growth

Some 4 weeks after tumour cells were injected intra-peritoneally, all animals were killed and tumour growth was scored semiquantitatively by two observers. Intraobserver variation was measured; variation never exceeded a score of 1 and affected the mean in less than one of 12 rats. Significant differences in tumour growth in the subcutis ($p<0.01$), omentum ($p<0.01$) and bowel anastomosis ($p<0.05$) were found between rats having conventional and laparoscopic small bowel resections. Differences in tumour growth at the other 5 abdominal sites were not significant. The total peritoneal tumourload of rats having conventional small bowel resection was significantly greater than the total peritoneal tumour load of rats having laparoscopic small bowel resection ($p<0.05$) (Table 1).

Comparison of tumour growth in rats undergoing laparoscopic small bowel resection and those having anaesthesia only, showed significantly more tumour growth in the scrotal fat ($p<0.02$) of rats having laparoscopic resections. Differences in tumour growth at the other six abdominal sites were not significant. The total peritoneal tumourload of all rats having a laparoscopic small bowel resection was significantly more than the total peritoneal tumour load of all rats having anesthesia only ($p<0.05$) (Table 1). Graphic presentation of total peritoneal tumour growth is shown in Fig. 1.

Groups / Sites	1	2	3	p-1 IvsII	p-2 IvsIII	p-3 IIvsIII
Subcutis	1.27±1.13	0.27±0.44	0.50±0.71	<0.01	ns	ns
Anastomosis	2.31±1.16	1.23±1.01		<0.05		ns
Omentum	2.38±1.24	1.12±1.00	0.38±0.52	<0.01	<0.01	ns
Scrotal fat	1.96±1.11	1.35±0.90	0.25±0.46	ns	<0.01	<0.02
Liver	0.92±0.76	0.85±0.80	0.25±0.46	ns	<0.05	ns
Retroperitoneum	1.81±1.47	1.15±0.90	0.56±0.62	ns	<0.05	ns
Peritoneum	1.27±0.93	0.85±0.55	0.44±0.62	ns	<0.05	ns
Kidney	1.85±1.20	1.23±0.93	0.38±0.52	ns	<0.01	ns
Total Tumourload	13.73±8.05	8.04±5.02	2.44±2.70	<0.05	<0.01	<0.05

Table 1. Mean peritoneal tumour growth (± s.d.) in rats undergoing conventional small bowel resection (group 1), laparoscopic small bowel resection (group 2) and rats undergoing anaesthesia only (group 3). P-value-1 represents differences between group 1 and 2, p-value-2 represents differences between group 1 and 3, and p-value-3 represents differences between group 2 and 3.

Experiment 2. Subrenal Capsule Assay

The weight of the subrenal tumour was measured 12 days after either conventional or laparoscopic small bowel resection or anesthesia only. Significant differences in mean (s.d.) tumour growth were found between the conventional and laparoscopic group 34.19(6.70) versus 23.31(5.27) mg respectively, ($p<0.01$) and between the conventional and anaesthesia group 34.19(6.70) versus 21.91(7.38) mg, ($p<0.01$). No significant differences in tumour growth were found between the laparoscopic and anaesthesia group (Fig. 2).

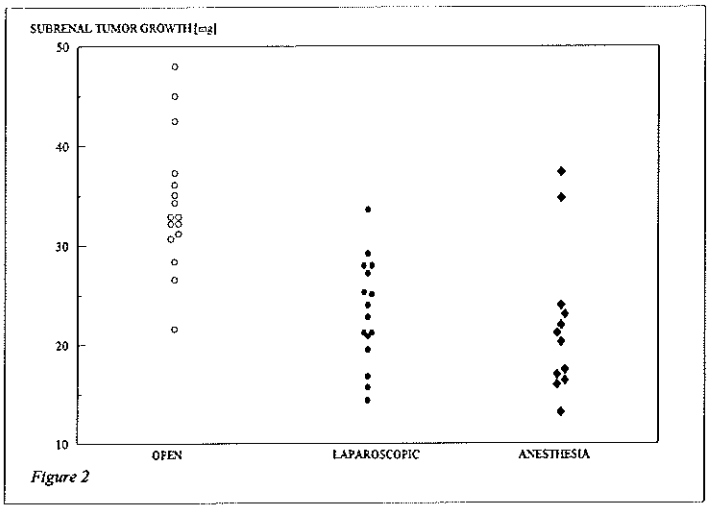

Figure 1. Graphic presentation of differences in total peritoneal tumour load of rats undergoing conventional small bowel resection (o), laparoscopic small bowel resection (•), and rats undergoing anaesthesia only (♦).

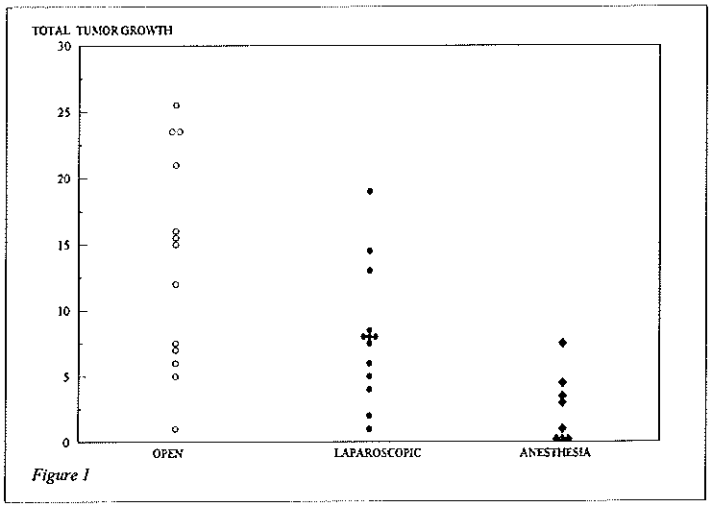

Figure 2. Graphic presentation of tumour weight in the subrenal capsule assay of rats undergoing conventional small bowel resection (o), laparoscopic small bowel resection (•), and rats undergoing anaesthesia only (♦).

Discussion

Stimulation of total peritoneal tumour growth was significantly less after laparoscopic surgery than conventional surgery. Analysis of the separate sites in the abdomen showed significant differences of tumour growth in the subcutis, bowel anastomosis, omentum and scrotal fat. Both the subcutis and bowel anastomosis are locations that are exposed to intense operative trauma resulting in production of growth factors and ischaemia. Growth factors have been shown to enhance not only wound healing but also the invasion and growth of cancer cells in vitro and in vivo[12,13]. Ischaemia reduces local defense mechanisms rendering the ischaemic site vulnerable for tumour implantation and growth[14]. Omentum and the scrotal fat are tissues on which tumour cells soil preferentially[8].

The subrenal capsule assay allows objective determination of tumour growth because the subcapsular tumour can be dissected easily and weighed accurately[15]. In the present study, subcapsular tumour growth was significantly greater in rats having conventional surgery than in those receiving laparoscopic surgery or anaesthesia only.

As rats are relatively inexpensive and detailed knowledge on their physiology is available, they were used to study the metabolic and oncological effects of laparoscopic surgery[16]. In a study that assessed post-operative body weight of rats after laparoscopic or conventional small bowel resection, those that had laparoscopic surgery regained their pre-operative body weight significantly earlier than rats which had an open surgery[8]. Laparoscopic surgery in rats therefore appears to be associated with less metabolic disturbances. Tumour take, as a parameter for growth of post-operative intraperitoneally implanted tumour cells, was assessed in an experimental study comparing rats having either laparoscopic or conventional small bowel resection. Significantly less tumour take was found in the laparoscopic group[8]. The finding of decreased tumour growth after laparoscopic surgery is in accordance with the report by Allendorf et al., who also found significantly less subcutaneous tumour growth after

insufflating the abdominal cavity without any operation in a murine model as compared with laparotomy[17].

The semiquantative analysis used in the present study to determine peritoneal tumour growth is controversial. Steller et al. analysed the results of various scoring methods for intraperitoneal tumour load. Visual assessment of tumour load, weighing the excised tumour and a radio-active method measuring uptake of 5-[^{125}I]-iodo-2'-deoxyuridine by the tumour tissue showed a linear correlation[18]. Therefore the most simple method, visual assessment, was chosen for the present experiment.

The decreased tumour growth after laparoscopic surgery could be due to several factors. The most important features of laparoscopic surgery are the use of minimal access and atraumatic tissue handling, resulting in less operative trauma than in conventional surgery. Busch et al. suggested that it was probably not blood transfusion that was causily related to the poor prognosis of colorectal cancer but the circumstances that necessitate transfusion, such as operative trauma[9]. Reduced operative trauma is also known to be associated with decreased attenuation of the immune defense mechanisms[19]. Although the role of the immune system in colonic cancer in humans is under debate, the weakly immunogenic tumour CC-531, which was used in the present study, is sensitive to immunomodulation[20]. Other evidence of improved immunological preservation after laparoscopic surgery was provided by Trokel, who reported in a rat model that delayed-type hypersensitivity is better preserved after laparoscopy than laparotomy[21]. Furthermore, Glaser et al. reported that laparoscopic cholecystectomy is associated with minor peroperative and postoperative metabolic stress response compared with conventional cholecystectomy[10].

In summary laparoscopic surgery in rats was associated with less peritoneal tumour growth and less tumour growth in the subrenal capsule assay than conventional surgery. In addition to the known favourable aspects of laparoscopic surgery, these results suggest strongly that reduced stimulation of tumour growth is inherent to minimally invasive surgery.

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5

Impact of Gas(less) Laparoscopy and Laparotomy on Peritoneal Tumor Growth and Abdominal Wall Metastases

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Abstract

Objective: A tumor model in the rat was used to study peritoneal tumor growth and abdominal wall metastases after carbon dioxide ($CO_{2)}$ pneumoperitoneum, gasless laparoscopy and laparotomy.

Summary Background Data: The role of laparoscopic resection of cancer is under debate. Insufflation of the peritoneal cavity with CO_2 is believed to be a causative factor in the development of abdominal wall metastases after laparoscopic resection of malignant tumors.

Methods: In the solid tumor model, a lump of 350 mg CC531 tumor cells was placed intraperitoneally in rats having CO_2 pneumoperitoneum (n=8), gasless laparoscopy (n=8) or conventional laparotomy (n=8). After 20 minutes the solid tumor was removed through a laparoscopic port or through the laparotomy. In the cell seeding model, $5x10^5$ CC531 cells were injected intraperitoneally prior to CO_2 pneumoperitoneum (n=12), gasless laparoscopy (n=12) or laparotomy (n=12). All operative procedures lasted 20 minutes. After 6 weeks, in the solid tumor model and after 4 weeks in the cell seeding model, tumor growth was scored semiquantitatively. All results were analyzed using the ANOVA analysis of variance.

Results: Peritoneal tumor growth in the CO_2 group was greater than in the gasless group ($p<0.001$). The size of abdominal wall metastases was greater at the port site of extraction of the tumor than at the other port sites ($p<0.001$). In the cell seeding model, peritoneal tumor growth was greater after laparotomy in comparison to CO_2 pneumoperitoneum ($p<0.02$). Peritoneal tumor growth in the CO_2 group was greater than in the gasless group ($p<0.01$). The port site metastases in the CO_2 group were greater than in the gasless group ($p<0.01$).

Conclusions: 1) direct contact between solid tumor and the port site enhances local tumor growth, 2) laparoscopy is associated with less intraperitoneal tumor growth than laparotomy, 3) insufflation of CO_2 promotes tumor growth at the peritoneum and is associated with greater abdominal wall metastases than gasless laparoscopy.

Keywords: Laparoscopy - Rat - Colon - Carcinoma - Metastasis - Wound recurrence

Introduction

Laparoscopic assisted colectomy has been shown to be technically feasible and associated with a low level of morbidity.[1,2,3] Until now, the total number of patients having laparoscopic colorectal resections is small in comparison to the wide extent of application of laparoscopic cholecystectomy.[4] The technical complexity, long duration and the high financial costs of laparoscopic colorectal procedures are probably responsible for the slow expansion of this type of surgery.[5] However, the most impeding factor seems the general concern about the feasibility of laparoscopic techniques to treat malignant disease.[6] More than twenty publications report 30 tumor recurrences at the site of cannula insertion or at the site of extraction (EXT) of the specimen.[7,8] The interval between operation and recurrence after laparoscopic resection of colorectal malignancies ranged from 3 to 26 months. Despite the fact that most of the reported cases have occurred after resection of either Dukes B, C or D disease, there also have been reports of metastatic spread of tumor after attempted curative resection of Dukes A lesions.[7]

Tumor recurrence in the abdominal wall after open surgery has been rarely reported. In a series of 1603 conventional resections of colorectal cancer, 11 patients developed abdominal wall metastases in the laparotomy incision or at the insertion site of a drainage tube, accounting for a rate of 0.6 %.[9] Although the true incidence of abdominal wall metastases after open resection of colorectal cancer is unknown, abdominal wall metastases after laparoscopic resections of colorectal cancer appear to be more prevalent. Thus, before the clinical use of laparoscopic techniques to remove malignant tumors, experimental studies are necessary to determine the effects of different operative approaches on tumour biology. We developed a solid tumor model in rats to study direct implantation of tumor cells in the extraction site. A cell seeding model was used to mimic the clinical situation of free viable intraperitoneal tumor cells.

The purpose of this study is to assess peritoneal tumor growth and abdominal wall metastases in rats after laparotomy, laparoscopy using carbon dioxide (CO_2),or gasless laparoscopy with mechanical elevation of the abdominal wall.

Materials and Methods

Animals

Male rats of the inbred WAG strain, weighing 200 to 300 g and aged 4 to 5 months were obtained from Harlan-CPB, Austerlitz, The Netherlands. Rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (Temperature 20-24°C, relative humidity 50% to 60%, 12 hours light-12 hours dark) and were fed a standard laboratory diet (Hope Farms, Woerden, The Netherlands) with free access to water and food before and after surgery. The protocols were approved by the Committee on Animal Research of the Erasmus University, Rotterdam, The Netherlands.

Tumor

CC-531 is a 1,2-dimethylhydrazine-induced, weakly immunogenic, moderately differentiated colon adenocarcinoma, transplantable in syngeneic WAG rats. This tumor is weakly immunogenic as determined by the immunization-challenge method of Prehn and Main[10]. The tumor was maintained in vitro in RPMI-1640 medium supplemented with 5 % fetal calf serum (virus and mycoplasm screened), 1% penicillin (5000 U/ml), 1% streptomycin (5000 U/ml) and 1% L-glutamine (200mM). All supplements were obtained from Gibco (Paisley, United Kingdom). Before their use, cells were trypsinized (5 minutes, 37°C), centrifuged (5 minutes, 3000RPM), resuspended in RPMI-1640 and counted. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). Viability always exceeded 95%. All tumor cells were injected within 4 hours after obtaining them.

To grow solid tumor, $1x10^8$ CC 531 tumor cells were injected in the right and left

flanks of syngeneic WAG rats. After 6 weeks the tumor volume in both flanks reached a volume of 2.5 cm^3 and the tumor mass was aseptically isolated with a scalpel from the outer membrane of the main lesion. Subsequently the tumor was cut into pieces of 350 mg, immersed in a culture solution, and stored at 4 °C until the solid lump was placed intra-abdominally. All lumps were placed intra-abdominally within 1-4 hours after collection of the solid CC 531 tumor from syngeneic WAG rats.

Operative Procedures

After being anesthetized with atropine 0.05 mg/kg s.c. (Centrafarm, Etten-Leur, The Netherlands), Domitor 0.25 mg/kg i.m. (Smithkline Beecham, Zoetermeer, The Netherlands), Ketalin 40 mg/kg i.p. (Apharmo, Arnhem, The Netherlands), the abdomen of the animals was shaved. The rat was secured to the operating table with adhesive tape in a supine position and the abdomen was cleaned with 70% alcohol and dried with gauze. The laparoscope, camera and attached cables were held at the desired angle by a flexible arm. The surgeon was sitting at one end of the operating table facing the video monitor. The instruments, trocars and laparoscope were cleaned with 70% alcohol before and after surgery.

The rats undergoing laparoscopy, had a 5-mm skin incision in the midline of the abdomen at two-third between the xiphoid process and the pubis. A 5-mm laparoscopic sheath with insufflation side port was introduced followed by introduction of a 4-mm arthroscope. Two other 5-mm ports were introduced under direct vision; one in the upper left quadrant (ULQ) and one in the upper right quadrant (URQ) of the abdomen. Rats which had a pneumoperitoneum were insufflated with CO_2 to a maximum pressure of 6 mm Hg, 10 minutes through an opened trocar in the ULQ and 10 minutes through the opened trocar in the URQ (6.4 liters CO_2 in total) to mimic normal turbulence wich occurs in clinical setting.

Mechanical elevation of the abdomen was established by 3 sutures attaching the trocars to a metal arm positioned over the rat. The trocar holes in both laparoscopy

groups were closed with one suture.

In the laparotomy group, a 5-cm abdominal skin incision was made in the midline of the abdomen. The abdomen was closed in one layer with a running suture. The operative time was 20 minutes in all procedures. To terminate anesthesia antisedan 2 mg/kg i.m. (Smithkline Beecham, Zoetermeer, The Netherlands) was given.

Experimental groups

Solid Tumor Model

Twenty-four rats participated in this experiment; 8 rats (group I) had a CO_2 pneumoperitoneum, 8 rats had mechanical elevation of the abdominal wall (group II) and 8 rats were subjected to a midline laparotomy (group III). In the laparoscopic groups, a lump of CC531 solid coloncarcinoma, weighing 350 mg was introduced through the EXT port in the upper left quadrant of the abdomen and placed between two lobes of the liver. An extra 5-mm site port was placed in the upper right quadrant (URQ). The 4 mm scope was introduced through a 5-mm port at the umbilicus (UMB).

In group I and II, the solid tumor was identified laparoscopically and grasped with a 5 mm dissection clamp after 20 minutes. The trocar at the EXT was withdrawn prior to extraction of the tumor. In group III, the lump was also placed between the liver, and removed after 20 minutes through the midline incision. All animals were sacrificed after 6 weeks. Abdominal wall metastases and intraperitoneal tumor-growth were scored semi-quantitatively. The scoring ranged from 0-5 per site and was assessed by two independent observers, according to the peritoneal cancer index described by Steller.[11] In case of disagreement between the two observers, the score was middled. A score of 0 indicated no presence of tumor, a score of 1 correlated with an estimated tumor diameter less than 0.5 cm, a score of 2 with a tumor diameter between 0.5 cm and 1 cm, a score of 3 with a tumor diameter between 1 cm and 2 cm, a score of 4 with a tumor diameter between 2 cm and 3

cm, and a score of 5 with a diameter exceeding 3 cm. Abdominal wall metastases were defined as tumor growth in the subcutis or peritoneum at the site of an abdominal scar. The total tumor load of each rat was defined as the total of tumor growth at all intra-abdominal sites (*e.g.*, kidney, liver, omentum, retroperitoneum and scrotal fat).

Cell Seeding Model

In this experiment, $5x10^5$ CC-531 tumor cells in 1.0 ml RPMI-1640 medium were injected intraperitoneally along the inner left and right abdominal walls before each procedure. To ensure that the cells were injected intraperitoneally, the drop test was performed previous to tumor cell injection. In this test, a drop of saline solution is placed within the open lumen of the injection needle. As the needle enters the peritoneal cavity, the relative negative intra-abdominal pressure sucks the fluid through the needle. The rats were randomized between three groups. Twelve rats (group IV) had CO_2 pneumoperitoneum, 12 rats (group V) had mechanic elevation of the abdominal wall and 12 rats were subjected to laparotomy (group VI). After 4 weeks all animals were sacrificed and tumor growth was scored semi-quantitatively as mentioned above. Each of the following sites was scored seperately: peritoneum and subcutis (=abdominal wall metastases), kidney, liver, omentum, retroperitoneum and scrotal fat. The total tumorload was defined as the sum of tumor growth at each site.

Statistics

The mean and standard deviation of the collected data were calculated. To analyze the data for significant differences, the analysis of variance was used. To assess the presence of a normal distribution of the data, a histogram was made. Results were considered statistically significant at $p<0.05$.

Results

Solid Tumor Model

In a histogram all results proved to be normally distributed. The results of assessment of abdominal wall metastases are shown in table 1a. All rats having either CO_2 or gasless laparoscopy developed abdominal wall metastases. Only 6 of the 8 rats having open surgery developed abdominal wall metastases. Significant differences of abdominal wall metastases were not found between the CO_2 pneumoperitoneum, gasless laparoscopy and laparotomy group at the site of extraction of the specimen. Comparison of the laparoscopic non-extraction sites (URQ versus UMB) also showed no significant differences. The size of the tumor at the extraction site (EXT) was significantly greater than the tumor size at the other two sites in both the CO_2 pneumoperitoneum group and the gasless laparoscopy group (both $p<0.001$) (table 1b).

Diffuse and extensive tumor growth was found in all rats. At the kidney, liver and scrotal fat, no significant differences were found after the three different operative procedures (table 1c). Tumor growth at the omentum and retroperitoneum in the gasless group was significantly less than in the laparotomy group and the CO_2 pneumoperitoneum group ($p<0.01$). Also total peritoneal tumor load in the gasless laparoscopy group was significantly less than in the laparotomy group and the CO_2 pneumoperitoneum group ($p<0.01$ and $p<0.001$).

Abdominal Sites	CO_2 (n=8)	Gasless (n=8)	Open (n=8)	p1 CO_2vs gasless	p2 CO_2vs open	p3 gasless vs open
Upper Right Q.	1.25±0.66	1.25±0.25	-	ns	-	-
Umbilicus	1.31±0.24	1.19±0.66	-	ns	-	-
Extraction site	2.25±0.38	2.38±0.35	1.81±0.75	ns	ns	ns

Table 1a. Mean diameter and standard deviation of the abdominal wall metastases at different trocar sites, and mean total tumorload and standard deviation of rats having CO_2 pneumoperitoneum (group I), gasless laparoscopy (group II) and laparotomy (group III) in the solid tumor model. The p-value 1 represents differences between group I and II; p-value 2 represents differences between group I and III; p-value 3 represents differences between group II and III.

Groups	URQ	UMB	EXT	p1 URQvs UMB	p2 URQvs EXT	p3 UMBvs EXT
CO_2	1.25±0.66	1.31±0.24	2.25±0.38	ns	<0.001	<0.001
Gasless	1.25±0.25	1.19±0.66	2.38±0.35	ns	<0.001	<0.001

Table 1b. Mean diameter and standard deviation of the abdominal wall metastases at different trocar sites of rats having CO_2 pneumoperitoneum (group I) and gasless laparoscopy (group II) in the solid tumor model. The p-value 1 represents differences between the tumor diameter at the trocar site in the upper right quadrant (URQ) and the trocar site at the umbilicus (UMB); p-value 2 represents differences between the URQ and the extraction trocar site in the upper left quadrant (EXT); p-value 3 represents differences in tumor diameter between the UMB and the EXT.

Abdominal Sites	CO2 (n=8)	Gasless (n=8)	Open (n=8)	p1 CO_2vs Gasless	p2 CO_2vs Open	p3 Gasless vs Open
Kidney	3.00±0.00	3.25±0.46	3.00±0.00	ns	ns	ns
Liver	2.50±0.53	2.63±0.52	2.75±0.71	ns	ns	ns
Omentum	2.50±0.93	1.38±0.52	2.38±0.52	<0.01	ns	<0.01
Retroperitoneum	2.75±0.46	1.25±0.46	2.50±0.76	<0.001	ns	<0.01
Scrotal fat	2.75±1.16	2.25±0.46	2.75±0.71	ns	ns	ns
Total Tumorload	13.50±1.2	10.75±1.04	13.13±1.20	<0.001	ns	<0.01

Table 1c. Mean tumor growth and standard deviation at different abdominal sites, and mean total tumor load and standard deviation in rats having CO_2 pneumoperitoneum (group I), gasless laparoscopy (group II) and laparotomy (group III) in the solid tumor model. The p-value 1 represents differences between group I and II; p-value 2 represents differences between group I and III; p-value 3 represents differences between group II and III.

Cell Seeding Model

In a histogram all results proved to be normally distributed. Tumor growth in rats after CO_2 pneumoperitoneum, gasless laparoscopy and laparotomy are shown in table 2.

Comparison of the CO_2 pneumoperitoneum group and the gasless group showed significantly greater tumor deposits at the peritoneum at the trocar sites ($p<0.01$). Furthermore after CO_2 pneumoperitoneum the tumor growth at the omentum ($p<0.001$), the scrotal fat ($p<0.02$) and the total tumor load ($p<0.001$) were greater than after gasless laparoscopy. Differences of tumor growth at the other 4 abdominal sites were not significant.

Comparing tumor growth in rats after either laparotomy or CO_2 pneumoperitoneum showed greater abdominal wall metastases in the laparotomy group as compared to the CO_2 pneumoperitoneum group (peritoneum $p<0.02$ and subcutis $p<0.001$). Differences of tumor growth at the other 5 sites were not significant. The total peritoneal tumor load of rats after laparotomy was significantly greater than the total peritoneal tumor load after CO_2 pneumoperitoneum ($p<0.001$).

Abdominal Sites	CO_2 (n=12)	Gasless (n=12)	Open (n=12)	p1 CO_2 vs Gasless	p2 CO_2 vs Open	p3 Gasless vs Open
Peritoneum	0.83±0.72	0.00±0.00	1.46±0.78	<0.01	<0.02	<0.001
Subcutis	0.17±0.39	0.00±0.00	1.25±1.08	ns	<0.001	<0.001
Kidney	1.58±0.63	1.25±0.58	1.96±1.10	ns	ns	ns
Liver	0.67±0.49	0.33±0.49	1.13±0.80	ns	ns	<0.01
Omentum	1.92±0.19	0.58±0.47	2.46±1.18	<0.001	ns	<0.001
Retroperit.	1.33±0.78	1.58±0.36	1.79±1.47	ns	ns	ns
Scrotalfat	1.50±0.95	0.50±0.60	1.92±1.08	<0.02	ns	<0.001
Total Tumorload	8.00±1.81	4.25±1.41	11.79±3.63	<0.001	<0.001	<0.001

Table 2. Mean tumor growth and standard deviation, and mean total tumor load and standard deviation at different abdominal sites in rats having CO_2 pneumoperitoneum (group IV), gasless laparoscopy (group V) and laparotomy (group VI) in the cell seeding model. The p-value 1 represents differences between group IV and V; p-value 2 represents differences between group IV and VI; p-value 3 represents differences between group V and VI.

Comparing gasless laparoscopy with open surgery showed greater abdominal metastases (peritoneum and subcutis $p<0.001$) in the open group. Tumor growth in the omentum, liver, scrotal fat and total tumor load were less after gasless laparoscopy (all $p< 0.01$).

Discussion

Tumor recurrence in the abdominal wall after conventional resection for colorectal cancer has been reported infrequently. In recent years, several reports of trocar site recurrence after laparoscopic oncological procedures have been published.[6,7,8] These reports have caused major turmoil because abdominal wall metastases had been rarely reported after conventional laparoscopic colorectal resection.[9] Abdominal wall metastases withhold many colorectal surgeons to use laparoscopic techniques to treat colorectal cancer[12]. Since the pathogenesis of abdominal wall metastases is unresolved, basic scientific studies focusing on this topic are indicated.

The mechanism of the development of abdominal wall metastases can be explained in various ways. First, direct implantation of tumor cells at the trocar or extraction site is considered to play a major role. Direct implantation at the trocar site can occur when a laparoscopic instrument has inadvertently grasped the tumor during the laparoscopic procedure. When this instrument is withdrawn through the trocar, tumor cells can detach, adhere to the trocar and implant at the trocar site during removal of the trocar at the end of the laparoscopic procedure. Extraction of the tumor through an small incision without protection of the abdominal wall appears to be the another variant of direct implantation of tumor cells.[13,14] Secondly, it can be hypothesised that insufflation of CO_2 in the peritoneal cavity causes turbulence displacing tumor cells. At the port sites, concentration of tumor cells occurs as a result of the "chimney effect": leakage of CO_2 alongside trocars causes a high local gas flow at the trocar sites. The high flow of CO_2 may contain aerosols with viable tumor cells, which results in implantation of tumor cells at these sites.[15] Free

intraperitoneal tumor cells occur frequently in digestive cancer as described by Juhl et al. who showed that 39 percent of patients operated for carcinoma of the stomach, colon, rectum and pancreas had free intraperitoneal malignant cells.[16] Spreading tumor cells, by manipulation or insufflation of gases into the peritoneal cavity appears to affect survival of patients with malignant intraperitoneal tumors. Zirngibl et al. reported that intra-operative tumor cell spillage reduced the 5-year survival rate after resection of rectal cancer from 70 to 44 percent.[17] The third mechanism of development of abdominal wall metastases is either hematogenous or lymphogenous migration of tumor cells to the abdominal wall. This seems unlikely because the blood and lymphatic vessels of the intra-abdominal organs lack an anatomical linkage with the abdominal wall. Moreover, some authors have reported port site metastases without any other evidence of metastases, suggestive of implantation metastasis.[18]

In this study, direct implantation of tumor cells at the extraction site was studied in the solid tumor model. Diffuse and extensive tumor growth was found after six weeks in all rats. It was clearly shown that extraction of an unprotected specimen without protection of the abdominal wall enhances tumor growth at the extraction site. To prevent tumor recurrence at the extraction site, the use of plastic bags or wound protectors is recommended to avoid direct contact between the tumor and the wound.[19] It is essential that extraction of the specimen is done through abdominal incisions wide enough to allow easy passage of the specimen. Furthermore, potentially traumatic manipulation of the tumor by laparoscopic clamps should be avoided by precise pre- or intra-operative localization of the tumor. This can be achieved using laparoscopic ultrasonography or tattooing of the lesion with Indian ink during colonoscopy prior to surgery.[20]

The cell seeding model in our study was used to mimic the clinical situation of free intraperitoneal tumor cells. Contrary to the solid tumor model in this experiment the rats were sacrificed after only four weeks, because tumor growth was extensive

after 6 weeks in the solid tumor model. To avoid difficult interpretation of tumor growth at separate sites, autopsy was done after 4 weeks. To assess the role of tumor growth induced by CO_2 insufflation, tumor growth was compared after gasless and CO_2 laparoscopy. In this study, tumor depositions at the trocar sites were significantly greater after CO_2 pneumoperitoneum in the cell seeding model. This finding is in accordance with the study by Jones et al. who found that the trocar site implantation tripled with the addition of pneumoperitoneum.[21] Therefore, turbulence in CO_2 laparoscopy appears to be an important factor in the pathogenesis of abdominal wall metastases.

In the cell seeding model in this study, the total tumor load was the greatest after open surgery and the smallest after gasless laparoscopy. A major difference between open and laparoscopic surgery is the degree of operative trauma. The extent of postoperative hormonal, metabolic and immunological changes is proportional to the degree of surgical trauma.[22] Although the influence of immunological and metabolic changes on tumor biology is unresolved in many cases, tumor growth appears to be proportional to the extent of operative trauma as well. Eggermont et al. showed in an experimental study that laparotomy promotes tumor growth.[23] Studies on tumor biology after either open and laparoscopic surgery have shown less tumor growth and less tumor take after laparoscopic surgery.[24] In an experimental study in mice by Allendorf et al. intradermal tumor growth was greater after open surgery in comparison to laparoscopic surgery.[25] In an earlier study in rats, we found less tumor take after laparoscopically assisted small bowel resection than after open small bowel resection.

The significant difference of tumor growth between CO_2 and gasless laparoscopy as found in this study deserves discussion. The operative trauma appears similar in these two techniques. However, the use of CO_2 resulted in greater peritoneal tumor growth. Increased absorption of CO_2 is known to be associated with systemic

effects such as acidosis, reduction in cardiac stroke volume and cardiac arrhythmia.[26,27] Direct effects of CO_2 on tumor growth are unknown, although a study by Watson et al. demonstrated that intraperitoneal macrophage activity was compromised after CO_2 insufflation as compared to insufflation with air and laparotomy.[28] The role of CO_2 in tumor biology demands further study. In our experiment, hypercapnia and acidosis were not monitored nor corrected. Therefore, it remains unresolved if either direct exposure of the peritoneum to CO_2 or CO_2-induced hypercapnia and acidosis promoted peritoneal tumor growth. Another factor which deserves analysisis are the duration and pressure of peritoneal CO_2 insufflation. Experimental studies assessing time and pressure dependency of CO_2-induced peritoneal tumor growth are necessary. Finally, use of other gasses than CO_2 should be evaluated.

Extrapolation of the results of this study to the clinical situation of laparoscopic resection of malignant tumors is limited for several reasons. First, injecting tumor cells in the peritoneal cavity is not similar to a localized colorectal cancer. Second, intraperitoneal surgical dissection was minimal and a bowel resection was not performed. Unfortunately, to our knowledge, it has not been possible until now to induce a localized colon cancer in experimental animals. However, recently we have developed in rats a laparoscopic model to remove kidneys with localized tumor. This model allows further study on the effects of gas(less) laparoscopy and laparotomy on tumor biology.

The favorable effects of laparoscopy on tumor growth in this study appear to contradict the great number of reports on ports site metastases after laparoscopic resection of colorectal cancer. However, extraction of colorectal cancers through narrow incisions, inadvertent grasping of the tumor and opening of the colon seem to have been major factors in these cases. In addition, the rate of abdominal wall recurrence after conventional colorectal cancer resections has received limited attention, and is therefore likely to be underreported.

Conclusion

This study showed that in surgery for intraperitoneal cancer the site of extraction of the specimen is at risk and deserves protection. The extraction site should allow easy passage of the specimen. Furthermore, laparoscopic surgery appears to be associated with less tumor growth than laparotomy, particularly when gasless techniques are used.

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Letter to the Editor

Dear Editor,

We read the paper written by Bouvy et al. with great interest[1]. They evaluated a very important issue of recent years on the effect of laparoscopic procedures in malignant diseases that was also demonstrated in some other clinical and experimental studies[2,3]. They stressed on the effect of CO_2 pneumoperitoneum on peritoneal tumor growth and abdominal wall metastases. A very important part of the peritoneal defense mechanisms is diaphragmatic clearance of particulate material, endotoxin, bacteria and probably the tumor cells via diaphragmatic lymphatics and thoracic duct.This part of peritoneal defense mechanisms can be easily effected by physiodynamic factors (e.g. intraabdominal pressure, obstruction of lymphatics)[4].

Neither this study nor the other experimental studies about laparoscopy and tumor growth deal with this probable aspect of tumor dissemination. We believe that it is relevant to take the lymphatic route of the peritoneal defense mechanisms into account. The tumor growth and adherence to the mediastinal lymph nodes and free tumor cells in peripheral blood might be evaluated.

We have also some comment on statistical analysis. The results of the present study have been analyzed with an inappropriate statistical method where the difference in tumor growth scores between groups was examined by ANOVA. To be able to do this, these scores have to cover the following presumptions[5]:

a. The "tumor growth score" variable must be a continious numerical variable.
b. The numerical variable has to be distributed normally in each group.
c. If the variable is numerical and normally distributed in each group, the variances shoul be similar among the groups (homogeneity of variances rule).

The "tumor growth score" variable in the mentioned article is an ordinal variable that is formed by recoding the "tumor diameter" variable. Though the authors inform that a histogram was made to show the presence of a normal distribution, one can calculate from the tables that the coefficients of variation range up to 87% that would not exceed 20% in normally distributed data. Furthermore, groups with less than 15 cases can not meet the characteristics of a normal distribution. Therefore, a nonparametric test would have been more appropriate for statistical analysis of the study.

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Authors reply

We thank Drs. Agalar, Daphan, Hayran and Sayek for their thorough commentary on our study: "The impact of gas(less) laparoscopy and laparotomy on peritoneal tumor growth and abdominal wall metastases".[1]

In their letter they state an interesting aspect of a peritoneal defense mechanism: diaphragmatic clearance via diaphragmatic lymphatics and the thoracic duct[2]. This clearance might be endangered in situations of elevated intraperitoneal pressure and might increase the spreading of endotoxin, bacteria and perhaps tumor cells through lymphatics. Further experimental investigations are mandatory to determine the relevance of the diaphragmatic lymphatic pathway. Several other dismal effects of intraperitoneal insufflation have also been suggested by other authors. Eleftheriadis et al. showed in an experimental study that intestinal ischaemia, free oxygen radical production, and increased bacterial translocation occurred in rats having a pneumoperitoneum[3]. Another aspect of increased intraperitoneal pressure is decreased blood flow in parietal and visceral peritoneum which renders it susceptible to tumor growth[4]. We think that elevated intra-abdominal pressure might play an important negative role in laparoscopy for malignant disease and deserves further study.

Furthermore, Agalar et al. suggest that our results were analyzed with an inappropiate statistical method. They argue that a nonparametric test would have been more suitable than analysis of variance (ANOVA) because our groups were relatively small. However, the principles of ANOVA are illustrated in the standard statistical textbook of Altman in 3 groups consisting of 8, 9 and 5 patients[5]. Hence, ANOVA may well be valid in small groups. Another reason to perform a nonparametric test would be that our data contained an ordening, but were possibly not really continuous and normally distributed. We therefore conducted the Mann-Whitney test. The results for table 1 and

2 are identical to our initial results obtained with ANOVA. Results for table 3 and 4 were slightly different and are shown with amendments from the original tables in bold. It is clear that the conclusions drawn earlier remain unchanged, either using the ANOVA or the non-parametric Mann-Whitney test.

Abdominal Sites	CO_2 (n=8)	Gasless (n=8)	Open (n=8)	p1 CO_2vs Gasless	p2 CO_2vs Open	p3 Gasless vs Open
Kidney	3.00±0.00	3.25±0.46	3.00±0.00	ns	ns	ns
Liver	2.50±0.53	2.63±0.52	2.75±0.71	ns	ns	ns
Omentum	2.50±0.93	1.38±0.52	2.38±0.52	**<0.02**	ns	**<0.001**
Retroperitoneum	2.75±0.46	1.25±0.46	2.50±0.76	<0.001	ns	**<0.001**
Scrotal fat	2.75±1.16	2.25±0.46	2.75±0.71	ns	ns	ns
Total Tumorload	13.50±1.2	10.75±1.04	13.13±1.20	<0.001	ns	**<0.001**

Table 3. Solid tumor model, results using the Mann-Whitney test

Abdominal Sites	CO_2 (n=12)	Gasless (n=12)	Open (n=12)	p1 CO_2 vs Gasless	p2 CO_2 vs Open	p3 Gasless vs Open
Peritoneum	0.83±0.72	0.00±0.00	1.46±0.78	**<0.001**	**<0.05**	<0.001
Subcutis	0.17±0.39	0.00±0.00	1.25±1.08	ns	**<0.01**	**<0.01**
Kidney	1.58±0.63	1.25±0.58	1.96±1.10	ns	ns	ns
Liver	0.67±0.49	0.33±0.49	1.13±0.80	ns	ns	<0.01
Omentum	1.92±0.19	0.58±0.47	2.46±1.18	<0.001	ns	**<0.01**
Retroperitoneum	1.33±0.78	1.58±0.36	1.79±1.47	ns	ns	ns
Scrotal fat	1.50±0.95	0.50±0.60	1.92±1.08	ns	ns	**<0.01**
Total Tumorload	8.00±1.81	4.25±1.41	11.79±3.63	**<0.01**	**<0.01**	**<0.01**

Table 4. Cell Seeding Model, results using the Mann-Whitney test

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Letter to the Editor

Dear Editor,

We read with interest the article of Bouvy et al[1]. Their study utilizes a rodent model to assess the impact of CO_2 and gasless laparoscopy as well as laparotomy on peritoneal tumor growth and abdominal wall metastases. The study demonstrates that laparotomy is associated with the greatest degree of postoperative tumour growth. Although laparoscopy results in less postoperative tumour burden, a difference is also noted depending on whether the laparoscopy is gasless or CO_2 based. In their discussion the authors reference a study of ours[2] relating to the effects of laparotomy, CO_2 laparoscopy and air laparoscopy on peritoneal immune response. In that study we have demonstrated that exposure of the peritoneal cavity to air regulates early inflammatory responses to surgery in a murine model and have identified that the most profound perturbations in postoperative proinflammatory responses occurred in the air laparoscopy and laparotomy study groups. Bouvy et al. have misinterpreted our data when they state that we identified that intraperitoneal macrophage activity was compromised after CO_2 insufflation. In fact, the presence of CO_2 in the peritoneal cavity was associated with preservation of macrophage function and CD11b receptor status. More importantly this study demonstrated, for the first time, that dysregulated peritoneal macrophage function in the air laparoscopy and laparotomy groups was attributable to factors in circulating air which appeared to induce endotoxin translocation both into the peritoneal cavity and systemic circulation. The study also implicated endotoxin as the factor responsible, as identical alterations in peritoneal immune function occurred in each of these groups. There is precedent to this, as Rylander et al[3] have previously reported that air contains small amounts of endotoxin (1 mg/m^3 approx).

We are interested in their findings in relation to intraperitoneal tumor growth. Studies carried out in our laboratory have similarly identified that breach of the peritoneal cavity through a laparotomy wound results in a significant increase in extraperitoneal primary tumor growth as well as increases in the number of hepatic and pulmonary metastases compared to CO_2 laparoscopy and anesthesia only. Increased tumor growth correlated with decreases in natural killer cell (NK) and lymphokine activated killer cell (LAK) function in the laparotomy and to a lesser extent CO_2 laparoscopy study groups[4]. More recent studies have gone on to demonstrate that air laparoscopy and direct injection of endotoxin into the peritoneal cavity result in increases in tumor growth similar to that seen with laparotomy[5]. Moreover work in progress in our laboratory has shown that near laparotomy (abdominal wound with no peritoneal membrane breach) with no peritoneal cavity exposure results in a negligible change in intraperitoneal immune function and postoperative extraperitoneal tumor growth. Of even greater interest is the finding that C3H-HeJ endotoxin hyporesponsive mice do not exhibit the early increase in postoperative tumor growth seen in their C3H-HeN counterparts, once again implicating endotoxin as a critical modeiator of not only the postoperative inflammatory response but also tumor growth. We believe that confirmation of these findings in the human will have significant implications in patients undergoing cancer surgery, specifically in terms of modulating micrometastatic tumor growth in the early postoperative period.

A number of studies have now suggested that the concept of the lesser trauma of minimal access surgery being responsible for lesser magnitudes of proinflammatory and immunoloical perturbations is unduly simplistic. The brilliant intuition of Joseph Lister that disease dust i.e. germs within the atmosphere was reponsible for wound infection heralded the modern era of surgery. We would propose that these organisms and their products are reponsible for phenomena previously attributable

to surgical trauma. Laparoscopic surgery has clearly demonstrated that beneficial modulation of the proinflammatory and immunological responses can occur. The total exclusion of air or the elimination of endotoxin from the open surgical field may bring similar benefits. The confirmation of refutation of this hypothesis in the clinical arena is clearly important.

H. P. Redmond

D. Bouchier-Hayes

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Authors reply

We thank Drs. Redmond and Bouchier-Hayes for their interesting commentary on our paper: "The impact of gas(less) laparoscopy and laparotomy on peritoneal tumor growth and abdominal wall metastases".[1]

It is obvious that the consequences of the interaction between CO_2 and macrophages on tumor defense mechanisms remain unsettled. In the study by Watson et al., the release of cytokines by macrophages was less after CO_2 insufflation than after air insufflation or laparotomy.[2] Cytokines are essential mediators in the activation of lymphocytes that possibly play a role in killing tumor cells. However, phagocytotic activity of macrophages was less attenuated by CO_2 than air or laparotomy in the study by Watson. Therefore, the macrophage activity appears to be compromised by CO_2 regarding release of mediators.

Jacobi et al. demonstrated that subcutaneous tumor growth was promoted by CO_2 insufflation as compared to helium and controls in a rat model.[3] It would be interesting to investigate the influence of other insufflation gases such as helium on the macrophage activity and tumor growth.

Earlier studies on tumor biology after either open and laparoscopic surgery have shown less tumor growth and less tumor take after laparoscopic surgery.[4] In an experimental study in mice by Allendorf et al.[5], intradermal tumor growth was greater after open surgery in comparison to laparoscopic surgery. For this reason it is interesting that Da Costa et al. demonstrated that endotoxin translocation is probably induced by factors in circulating air. In this study direct injection of endotoxin into the peritoneal cavity resulted in increases in intraperitoneal tumor growth similar to laparotomy[6]. To us it seems a very interesting statement that endotoxin is a critical mediator, not only in postoperative inflammatory response but also in tumor growth.

In our opinion it remains unresolved if either direct effects of CO_2, exposure of the peritoneum to endotoxins, or the elevated intra-abdominal pressure itself, which causes local ischaemia and bacterial translocation, is the main cause to promote peritoneal tumor growth more after CO_2 insufflation than after gasless laparoscopy. Experimental studies assessing time and pressure dependency of CO_2-induced peritoneal tumor growth, the use of other gases than CO_2 , endotoxin free gases and the influence of elevated intraperitoneal pressure are necessary to evaluate the different aspects of laparoscopy on tumor growth.

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Letter to the Editor

Dear Editor,

We read with interest the article of by Bouvy et al. and wish to congratulate them with this fine study[1].

In their cell seeding model, using a semiquantitative peritoneal cancer index by Steller, they state that intraperitoneal tumor load was significantly greater after laparotomy compared to laparoscopy and that tumor depositions at the trocar sites were significantly greater after CO_2 pneumoperitoneum.

In a similar study using the peritoneal cancer index of Eggermont and a cancer index of Chauffert we found no difference in intraperitoneal tumor load when 4 groups of rats were compared (intraperitoneal CC531 cells only, laparatomy, CO_2 pneumoperitoneum, CO_2 pneumoperitoneum and "ports")[2]. However when trocar ports were introduced we also noticed a redistribution of intraperitoneal tumor growth towards trocar sites. Interestingly, differeces were noticed in tumor score of the 4 groups when both scoring systems were compared. In the group were tumor load was most important according to the Eggermont index, this was not the case when the Chauffert index was used. In our view this illustrates the relative inadequacy of accurately quantifying the intraperitoneal tumor load using these scoring systems. Perhaps the total tumor weight gives a better idea of the intraperitoneal tumor load.

The authors also stated that .."to their knowledge it has not been possible to induce a localized colon cance rin experimental animals". We recently developed a localized colon cancer model in rats, by inducing the growth of an adenocarcinoma at an anastomotic site[3]. We are currently investigating the effect of CO_2 pneumoperitoneum on the development of trocar metastases in this model.

G. Hubens

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Authors reply

We thank Dr. Hubens for his valuable commentary on our paper: "The impact of gas(less) laparoscopy and laparotomy on peritoneal tumor growth and abdominal wall metastases"[1].

Indeed the conclusion is interesting that, based on the results of the study of Hubens et al., both the Eggermont-index and the Chauffert-index to score intraperitoneal tumor load as used in their study, showed different statistical results[2]. However, the statement of Hubens that use of total tumor weight to measure intraperitoneal tumor growth seems superfluous, as indicated by Steller[3]. In the study by Steller, similar results were obtained when visual scores (peritoneal cancer index), tumor mass and 125IUdR tumor uptake were assessed. Steller proved in his thesis that either of these three assessments as mentioned above can be employed for quantifying intraperitoneal tumor load, since no significant diferences between the methods were found. Chauffert's method involves assessment of the presence of tumor noduli smaller than 1 mm (index 1), tumor noduli greater than 1 mm without ascites (index 2) and the presence of ascites with diffuse carcinomatosis (index 3)[4]. The disadvantage of the Chauffert method is that it classifies tumor presence for the entire peritoneal cavity in 4 categories while the Eggermont method can be used for separate peritoneal sites, and uses 6 different categories. Therefore, we consider the Eggermont method (peritoneal cancer index) more feasible and accurate in tumor experiments.

We congratulate Hubens and his colleagues on developing a localized colon cancer model in the rat. This model will allow further experimental studies.

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Port Site Metastases: Role of local Ischemia and Gas Leakage

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Abstract

Background: Port site metastases after laparoscopic procedures in patients with digestive malignancies have evoked concern. The pathogenesis of port site metastases remains unclear. Two experiments in rats were performed to determine the impact of both local ischemia and leakage of CO_2 along trocars (chimney-effect) in the development of port site metastases.

Methods: Experiment I: 10 WAG rats had four 5 mm incisions in all abdominal quadrants. The incisions on the right side were crushed to induce ischemia. After inserting 5 mm trocars in all incisions, a pneumoperitoneum was created and CC-531 tumor cells were injected intraperitoneally. CO_2 was insufflated for 20 minutes. Experiment II: 10 WAG rats had a 5 mm incision in the left and a 10 mm incision in the right abdominal upper quadrant; 5 mm trocars were inserted in both incisions. After insufflating the abdomen, CC-531 tumor cells were injected intraperitoneally. Total leakage of CO_2 along the trocar in the right quadrant was 10 liters. After 4 weeks, in both experiments the tumor deposits at the trocar sites were assessed. Statistical analysis was performed by the Wilcoxon matched-pairs test.

Results: Experiment I: Median weight of tumor deposits at the trocar sites without induced ischemia was 22 mg. At the ischemic port sites, median weight of tumor deposits was 316 mg ($p = 0.007$). Experiment II: Median weight of tumor deposits at the leaking trocar sites was 175 mg and at the control sites 67 mg ($p = 0.005$).

Conclusion: Local ischemia at trocar sites and leakage of CO_2 along a trocar appear to promote implantation and growth of tumor cells at port sites.

Keywords: Port Site - Metastasis - Laparoscopy - Cancer - Chimney - Ischemia

Introduction

Laparoscopic surgery is well accepted in the treatment of a variety of benign disorders. Several authors have shown that the laparoscopic technique is as well feasible and safe in malignant diseases, and associated with less blood loss and reduced morbidity compared to open surgery[5,6]. However, numerous case reports of port site recurrences following diagnostic laparoscopy or laparoscopic resections in patients with malignancies have been published, which have caused great concern[14,19]. Therefore, the laparoscopic approach to malignant disease remains controversial.

Until now, the pathogenesis of port site metastases is not well understood. The most likely mechanism seems to be implantation of tumor cells in trocar wounds during operation. Metastatic growth at port sites that were not used for specimen extraction suggest that factors other than direct contact with the resected specimen are responsible. One factor that possibly puts the trocar site at risk for tumor growth is local ischemia. The traumatized trocar wound appears a good medium for implantation and growth of tumor cells, because growth factors are abundantly present in traumatized tissue[15]. Another factor may be the leakage of CO_2 along a trocar, the so called 'chimney-effect'[13]. Leakage around a trocar could result in high local flow containing free floating tumor cells. These hypotheses require the presence of free viable tumor cells in the peritoneal cavity and transportation of these cells to the trocar wounds.

This study was designed to determine the role of both trauma of the trocar wounds and gas leakage along a trocar on tumor growth at the port sites.

Material and Methods

Animals

Twenty male pathogen-free WAG/Rij rats weighing 200 to 250 g, obtained from Harlan-CPB, Austerlitz, The Netherlands, were used in this study. The animals were housed in free-standing cages under standard laboratory conditions (temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark) and were fed a standard laboratory diet (Hope Farms, Woerden, The Netherlands) with free access to water and food before and after surgery. The protocols were approved by the Committee on Animal Research of the Erasmus University, Rotterdam, The Netherlands.

Tumor

CC-531 is a 1,2-dimethylhydrazine-induced, weakly immunogenic, moderately differentiated colon adenocarcinoma, transplantable in syngeneic WAG rats. The tumor was maintained *in vitro* in RPMI-1640 medium supplemented with 5% fetal calf serum (virus and mycoplasm screened), 1% penicillin (5000 units/ml), 1% streptomycin (5000 units/ml) and 1% L-glutamine (200 mmol). All supplements were obtained from Gibco (Paisley, UK). Before their use, cells were trypsinized (5 minutes, 37°C), centrifuged (5 minutes, 3000 rpmg), resuspended in RPMI-1640 and counted. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% sodium chloride solution). Viability always exceeded 95%. All tumor cells were injected in the rats within 5 hours after obtaining them.

Surgical procedures

Operations were performed under general inhalation anesthesia using ether. After shaving the abdomen, the rat was secured to the operating table with adhesive tape in a supine position and the abdomen was cleaned with 70% alcohol. The laparoscopic equipment, provided by Karl Storz Endoscopes and Duffner, was adjusted to the rat.

The laparoscope (diameter 4 mm), trocars (diameter 5 mm) and instruments were cleaned with 70% alcohol before and after surgery.

Ischemia Model

In 10 rats four 5 mm incisions were made in all abdominal quadrants. The incisions on the right side were subjected to a standard injury by placing two traumatic clamps on both the cutaneous and musculoperitoneal layer during 10 minutes to induce ischemia. Subsequently, 5 mm trocars were inserted in all four incisions and a purse string suture (Vicryl® 3-0, Ethicon, Sommerville, NJ, USA) was placed to prevent leakage of gas around the port. After creating a pneumoperitoneum with CO_2 to a maximum pressure of 6 mmHg, absence of leakage was checked by the insufflator. Under laparoscopic vision 5 x 10^5 CC-531 tumor cells in 1 ml RPMI-1640 medium were injected through the midline and equally distributed in the four quadrants of the peritoneal cavity. CO_2 was insufflated through each trocar during 5 minutes. After 20 minutes all trocars were removed at the same time while the peritoneal cavity was still insufflated to prevent that the trocars would touch the abdominal contents. The incisions were closed in one layer with interrupted 2-0 silk sutures (B.Braun, Melsungen AG, Germany). Four weeks later the animals were sacrificed, and tumor deposits at all trocar sites were precisely excised and weighed.

Gas Leakage Model

In 10 rats a 5 mm trocar was inserted through a 5 mm incision in the left upper quadrant of the abdomen. The trocar was secured with a purse string suture (Vicryl® 3-0) to prevent leakage of CO_2. After creating a pneumoperitoneum with CO_2, absence of leakage was checked by the insufflator. Subsequently, the abdomen was desufflated. In the right upper quadrant of the abdomen a 10 mm incision was made and a 5 mm trocar was inserted. A pneumoperitoneum was established with CO_2 to a maximum pressure of 6 mmHg and under laparoscopic vision 5 x 10^5 CC-531 tumor cells in 1 ml RPMI-1640 medium were injected through the midline and equally distributed in

the peritoneal cavity. Total amount of leakage of CO_2 along the trocar in the right upper quadrant was set at 10 liters (15-20 minutes). All trocars were removed at the same time while the abdomen was still insufflated. The incisions were closed in one layer with interrupted 2-0 silk sutures (B.Braun). After 4 weeks an autopsy was done and tumor deposits at all trocar sites were resected and weight was measured.
The Wilcoxon matched-pairs test was used to analyze the data for significant differences. Statistical significance was achieved at $p < 0.05$.

Results

Ischemia Model

One rat in the ischemia model died from anaesthesia causes. In the ischemia model, all rats showed tumor take in the abdomen at autopsy. The median weight of tumor deposits at the trocar sites without induced ischemia was 21.6 mg (0-192.5 mg). At the ischemic port sites, median weight of tumor deposits was 315.9 mg (1.4-748.7 mg). Tumor growth at the traumatized trocar sites was in all cases greater than at the control trocar sites. The median difference was 284.1 mg (1.4-716.8 mg) which is significant ($p = 0.008$). (Fig. 1)

Gas Leakage Model

In all rats tumor take was found in the abdomen. All rats had greater abdominal wall metastases at the port sites with leakage along the trocar in comparison to the not leaking trocar sites. The median weight of tumor deposits at the leaking port sites was 175.3 mg (34.1-556.9 mg) and at the control trocar sites 67.1 mg (9.8-182.7 mg). The median difference was 111.9 mg (24.3-374.2 mg) which is significant ($p = 0.005$). (Fig. 2)

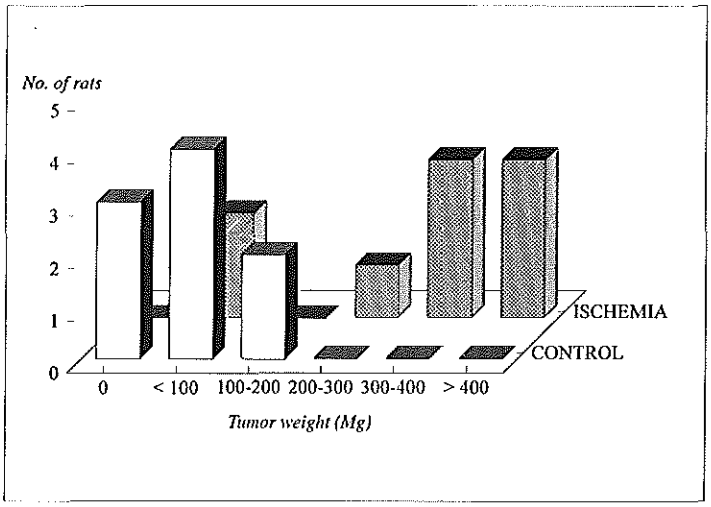

Figure 1. Distribution of tumor weight at the ischemic trocar sites and control sites.

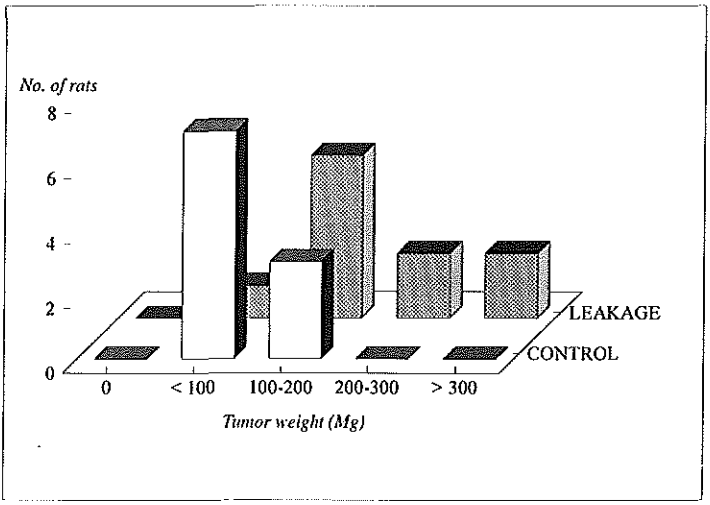

Figure 2. Distribution of tumor weight at the leaking trocar sites and control sites.

Discussion

Tumor growth is enhanced by surgery, probably due to tumor manipulation and transient immunosuppression[4]. Laparoscopic surgery causes less tissue trauma in comparison to open surgery which appears to be associated with less tumor growth and tumor take as shown in experimental studies[1,2]. However, tumor recurrence in abdominal wounds has been described after laparoscopy. The true incidence of wound recurrences following laparoscopy is unclear. At least 30 port site metastases after laparoscopic colorectal operations have been reported and incidences vary from 0 to 21%[14,19]. Wexner *et al.* estimated, based on all presented and reported cases, the incidence of port site metastases to be 4%[19]. In contrast, wound recurrence following conventional treatment of colorectal cancer is thought to be uncommon but is possibly underestimated. Hughes et al. and Reilly et al. reported incidences of abdominal wall recurrences after laparotomy for colorectal cancer of 0.8% and 0.6% respectively[11,18], but others report a more frequent occurrence varying from 3.3 to 5.3%[7]. The development of port site metastases is probably multifactorial and a number of possible mechanisms have been postulated. These mechanisms will be discussed separately.

Direct implantation of tumor cells.

Extraction of colorectal cancer through narrow incisions has been shown to be associated with high incidence of wound recurrence at the extraction site by contact between the tumor and the wound[16]. Direct implantation leading to recurrence of malignancy at trocar sites can also be caused by contaminated instrument-to-trocar contact. Tumor cells can stick to instruments and subsequently adhere to a trocar during withdrawal of the instruments, possibly leading to implantation of tumor cells at the trocar site when the trocar is removed. This has been confirmed by Hewett et al., who identified malignant cells in several washings of laparoscopic instruments and trocar wounds[9].

Gas or fluid leaking along a trocar, could act as vehiculum transporting tumor cells to the trocar wound, the so-called 'chimney effect'[13]. In the 'gas leakage' model we observed a significant rise in tumor weight at the 'leaking' ports sites, suggesting such a mechanism. Based on this experiment we are unable to differentiate aerosol leakage from leakage of fluid containing tumor cells as a causative factor in development of wound metastases. Whelan et al. failed to detect the presence of tumor cell containing aerosols in a high-pressure CO_2 environment[20]. However, they proved that under turbulent conditions such as rapid desufflation, tumor cells in liquid suspension can be transported *in vitro*. In our experiment, desufflation was performed by simultaneous removal of both trocars in order to prevent trocar contact with abdominal contents during desufflation. Either the leakage during the period of pneumoperitoneum or an increased leakage at the site of the bigger trocar site during desufflation is responsible for an increased tumor mass at the site, proving the possibility of transportation of tumor cells through leakage *in vivo*.

Trauma and ischemia.

It is well known that local factors influence the site and growth of metastases[17]. Murthy et al. described the possibility of tumor cell entrapment in the clot formation in fresh wounds[15]. Such clot formation could not only bind tumor cells but also offers nutrition and a barrier against host defense mechanisms. Other studies indicate that malignant cells grow more rapidly in areas of high cellular proliferation, such as regenerating tissue, mediated by host generated growth factors[8]. Failing host defense mechanisms and the presence of tumor necrosis factor as well as the transient immunosuppression during surgery may all contribute to the enhancement of tumor adherence and growth in areas of tissue trauma. Accordingly, in our ischemia model we observed significantly higher tumor weight at the trocar sites that had been subjected to ischemic trauma.

CO_2 influence

Some reports suggest a direct influence of carbon dioxide on tumor growth. In several

experiments, Jacobi et al. showed that insufflation of CO_2 promoted tumor growth compared with helium and control in a rat model[12]. In our own laparoscopic experiments with rats, we found that tumor growth was significantly greater in a CO_2 insufflation group compared to a gasless group[3]. However, Hubens et al. failed to demonstrate any effect of CO_2 pneumoperitoneum on tumor cell implantation or growth[10]. Therefore, the direct effects of carbon dioxide on tumor growth require further study.

A better understanding of the mechanisms contributing to port site metastases is necessary to be able to prevent them. Many authors have already advocated important preventive measures such as enlarging extraction sites, protection of the extraction wound, the use of laparoscopic specimen bags and less traumatic tissue handling. Leakage along trocar openings can simply be overcome with a purse string suture around the trocar. Furthermore it seems wise to prevent trocar wound ischemia by making the trocar incision not too small. It can be argued that a purse string suture in itself induces ischemia and in that way can lead to enhancement of tumor adherence and growth. However, the fact that a significant greater tumor growth was seen at the site of the leaking trocar then on the not leaking purse string site suggest that leakage contribute more to tumor growth then local ischemia induced by a purse string. However, the use of balloontrocars can prevent both disadvantages of leakage and of local ischemia.

Clearly the occurrence of port site tumor implantation and growth is a complex process in which many factors are involved. Hopefully, the elucidation of contributing factors will help us to overcome this serious problem in laparoscopic surgery for malignancy.

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Effects of CO_2 Pneumoperitoneum, Air Pneumoperitoneum and Gasless Laparoscopy on Body Weight and Tumor Growth

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Archives of Surgery, in press.

Abstract

Objective: Body weight and tumor growth were studied in rats after CO_2 pneumoperitoneum, air pneumoperitoneum and gasless laparoscopy.

Summary Background Data: The oncological consequences of intraperitoneal CO_2 insufflation during laparoscopic resection of cancer are under debate. The impact of other insufflating gasses or gasless laparoscopy on cancer requires study.

Methods: On day 1, a lump of 8 mg ROS-1 tumor was placed under the renal capsule of both kidneys in rats. In experiment I, rats had either CO_2 insufflation (n=10) or gasless laparoscopic bowel resection (n=10) on day 3 and were sacrificed after 7 days. In experiment II, rats had either laparoscopic bowel resection with CO_2 insufflation (n=11) or insufflation with air (n=11) on day 3 and were sacrificed after 7 days. In both experiments postoperative weight loss and tumor growth were measured. Differences in body weight and tumor growth were tested with analysis of covariance.

Results: Renal subcapsular tumor growth in the gasless laparoscopy group was less than in the CO_2 pneumoperitoneum group (p=0.038). Postoperative weight loss in rats that had CO_2 insufflation and gasless laparoscopy showed no differences (p=0.55). No differences in tumor growth nor weight loss were found between rats that had insufflation with CO_2 and insufflation with air (p=0.61 and p=0.68).

Conclusions: Restoration of body weight after laparoscopic surgery was similar after either CO_2, air or gasless laparoscopy. Gasless laparoscopy was associated with less renal subcapsular tumor growth than insufflation with CO_2. Therefore, application of gasless techniques in laparoscopic oncologic surgery demands further study.

Keywords: Laparoscopy - rat - carcinoma - gasless - CO_2 - air - pneumoperitoneum - body weight.

Introduction

Laparoscopic techniques are progressively utilized in surgical practice for both benign and malignant disease. Although minimally invasive surgery has become the preferential approach to gallbladder stone disease, hiatal esophageal disorders and small adrenal tumors, the value of minimally invasive techniques in the treatment of malignant disease remains unresolved[1,2,3,4]. Major concern has been caused by more than twenty publications reporting tumor recurrences at the site of cannula insertion or at the site of extraction of the specimen after laparoscopic resection for cancer[5,6]. However, experimental studies have shown that tumor take and growth are less after laparoscopic surgery than after open surgery[7,8]. This apparent oncological benefit of laparoscopic surgery has been attributed to reduced surgical trauma which is associated with laparoscopic surgery. However, one of the drawbacks of laparoscopic surgery is that intraperitoneal insufflation of CO_2 is required to create a working space. CO_2 insufflation of the peritoneal cavity results in hypercarbia, acidosis, hemodynamic alterations and gut ischemia[9,10]. The metabolic and oncologic consequences of CO_2 pneumoperitoneum have not been studied extensively until now. Alternatives to CO_2 pneumoperitoneum are use of other insufflation gasses or mechanical elevation of the abdominal wall (gasless laparoscopy). The effects of CO_2 and increased abdominal pressure on cancer are unknown and demand further study.

The aim of this study is to assess bodyweight and tumor growth after intraperitoneal insufflation of CO_2 compared to air or gasless laparoscopy.

Materials and Methods

Animals

Thirty-four male inbred WAG-Rij strain rats, weighing 260-330 g and aged 3-4

months, obtained from Harlan-CPB (Austerlitz, the Netherlands) were used. The rats were bred under specific pathogen-free conditions. The animals were housed in free-standing cages and acclimated to standard laboratory conditions (Temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark). The rats were fed a standard laboratory diet (Hope Farms, Woerden, the Netherlands) with free access to water and food before and after surgery. The experimental protocols adhered to the rules in the 'Dutch Animal Experimental Act' (1977) and the 'Guidelines on the protection of Experimental Animals' published by the council of the EC (1986). The protocol was approved by the 'Committee on Animal Research' of the Erasmus University, Rotterdam, The Netherlands.

Tumor

The ROS-1 osteosarcoma (transplantable to WAG/Rij rats) was used. This osteosarcoma originated spontaneously in the tibia of a rat[11]. The tumor was maintained in vitro in RPMI 1640 medium supplemented with 5 % fetal calf serum (virus and mycoplasm screened), 1% penicillin (5000 U/ml), 1% streptomycin (5000 U/ml) and 1% L-glutamine (200mM). All supplements were obtained from Gibco (Paisley,United Kingdom). Before their use, cells were trypsinized (5 minutes,37°C), centrifuged (5 minutes, 3000RPM), resuspended in RPMI 1640 and counted. Viability was measured with trypan-blue exclusion (0.3% in a 0.9% NaCl-solution). Viability always exceeded 95%. To grow solid tumor $2x10^6$ ROS-1 tumor cells were injected in the right and left flanks of syngeneic WAG rats. After 3 weeks the tumor volume in both flanks reached a volume of 2.5 cm^3 and the tumor mass was aseptically isolated with a scalpel from the outer membrane of the main lesion. Subsequently the tumor was cut into 1 mm^3 cubes of about 8 mg, immersed in a culture solution, and stored at 4 °C until the solid lump was placed under the renal capsule. All cubes were placed underneath the renal capsule, within 1-4 hours after collection of the solid ROS-1 tumor from syngeneic WAG rats.

Operative Procedures

After being anesthetized with Atropine 0.05 mg/kg (Centrafarm, Etten-Leur, The Netherlands), Domitor 0.25 mg/kg i.m. (Smithkline Beecham, Zoetermeer, The Netherlands), Ketalin 40 mg/kg i.p. (Apharmo b.b., Arnhem, The Netherlands), the abdomen of the animals was shaved. The rat was secured to the operating table with adhesive tape in a supine position and the abdomen was cleaned with 70% alcohol and dried with gauze.

On day one, all rats underwent a two and a half centimeter midline laparotomy. Both kidneys were exposed and 8 mg of solid ROS-1 tumor was placed under the capsule of both kidneys under microscopic vision. During operation, the viscera were covered with phosfate-buffered saline (PBS) wetted gauze, as mentioned above. The operative time of this procedure varied between 20 and 25 minutes. Viscera were returned to the abdominal cavity and the abdomen was closed in one layer by a running suture.

On day three, all rats were weighed and underwent a laparoscopic procedure. The laparoscope, camera and attached cables were held at the desired angle by a flexible arm. The surgeon was sitting at one end of the operating table facing the video monitor. The instruments, trocars and laparoscope were cleaned with 70% alcohol before and after surgery. All rats had a 5 mm skin incision in the midline of the abdomen at two-third between the xiphoid process and the pubis. A 5 mm laparoscopic sheath with insufflation side port was introduced followed by introduction of a 4 mm arthroscope. Two other 5 mm ports were introduced under direct vision; one in the upper left quadrant and one in the upper right quadrant of the abdomen. Rats which had a pneumoperitoneum were insufflated with CO_2 or air (O_2 21%, N_2 79%) to a maximum pressure of 6 mm Hg during 20 minutes (average 7.5 L in total). Mechanical elevation of the abdomen was established by 3 sutures attaching the trocars to a metal arm positioned over the rat. In all laparoscopic procedures, a small bowel resection (4 cm in lenght) followed laparoscopic extraction of an 8 cm segment of the ileum. Anastomosis was performed

extracorporeally by a silk 7-0 running suture. All trocar holes were closed with one 2-0 silk suture. Resection time ranged from 25 from 35 minutes.
To terminate anesthesia Antisedan 2 mg/kg i.m. (Smithkline Beecham, Zoetermeer, The Netherlands) was given.

Experiment I. CO_2 pneumoperitoneum versus gasless laparoscopy
On day one, twenty-two rats had a two and a half centimeter midline laparotomy and 8 mg of solid ROS-1 tumor was placed under the capsule of both kidneys under microscopic vision, as mentioned above. Two days later 10 rats (group I) had a CO_2 pneumoperitoneum and 10 rats (group II) had gasless laparoscopy. A small bowel resection was performed in both groups, as described above. Seven days after tumor implantation all animals were sacrificed, body weight loss was measured (by subtracting the weight on day 3 from day 7) and the growth of the subcapsular tumor was measured by weighing the enucleated lump on day 7 and subtract this weight from the implanted tumor weight on day 1.

Experiment II. CO_2 pneumoperitoneum versus air pneumoperitoneum
On day one, twenty-two rats underwent a two and a half centimeter midline laparotomy and both kidneys were exposed and 8 mg of solid ROS-1 tumor was placed under the capsule of both kidneys under microscopic vision. Two days later, 11 rats (group III) were insufflated with CO_2 pneumoperitoneum and 11 rats (group IV) had air pneumoperitoneum for a period of 20 minutes. A small bowel resection (4 cm in lenght) followed laparoscopic extraction of a 8 cm segment of the ileum. Anastomosis was performed extracorporeally, as described above. Seven days after tumor implantation all animals were sacrificed, body weight loss was measured (by subtracting the weight on day 3 from day 7) and the growth of the subcapsular tumor was measured by weighing the enucleated lump on day 7 and subtract this weight from the implanted tumor weight on day 1.

Statistics

The mean and the standard deviation (SD) of the collected data were calculated. To test for significant differences, analysis of covariance was used[12]. This analysis assumes a normal distribution, which was tested with the Kolmogorov-Smirnov test (SPSS Inc., Chicago, Illinois). In absence of a normal distribution, data were transformed logarithmically. Results were considered statistically significant at a two-sided p-value of less than 0.05.

Results

Experiment I. CO_2 pneumoperitoneum versus gasless laparoscopy

One rat in each group died of anesthetical causes. Body weight proved to be normally distributed. No significant differences in post-operative weight loss were found between rats that had laparoscopic small bowel resection with either CO_2 pneumoperitoneum or gasless laparoscopy (Table 1). Figure 1 shows the tumor growth underneath the renal capsule from day 0 (implantation), day 3 (intervention) until day 7 (obduction) for each group (I-IV). The tumor growth was statistically significant within all groups. The distribution of tumor weight was not normal. A logarithmical transformation was therefore applied. Significant differences in tumor growth were found between CO_2 laparoscopy (group I) and gasless laparoscopy (group II)(Table 1).

N=20	*CO_2*	*Gasless*	*p-value*
post-operative body weight loss[%]	7.04 ± 1.53	5.85 ± 1.75	0.55
subrenal tumor growth [mg]	16.87 ± 5.72	10.20 ± 6.51	0.038

Table 1. Mean percentage of body weight loss and mean subrenal tumor growth (± SD) of rats having CO_2 pneumoperitoneum and gasless laparoscopy (experiment I).

Experiment II. CO_2 pneumoperitoneum versus air pneumoperitoneum

Post-operative body weight loss showed a normal distribution. No significant differences in post-operative weight loss were found between CO_2 pneumoperitoneum (group III) and air pneumoperitoneum (group IV), Table 2. A logarithmical transformation was applied to analyze tumor weight because tumor weight distribution was not normal. No significant differences in tumor growth were found between laparoscopic bowel resection with CO_2 pneumoperitoneum (group III) and air pneumoperitoneum (group IV), Table 2.

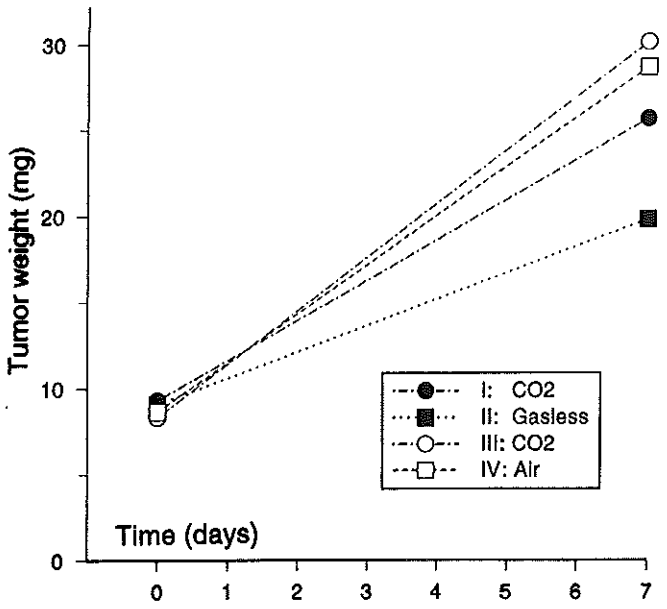

Figure 1 Mean tumor growth underneath the renal capsule from day 0 (implantation), day 3 (intervention) untill day 7 (obduction) for each group (I-IV).

N=22	*CO_2*	*Air*	*p-value*
post-operative body weight loss[%]	4.12 ± 1.63	4.55 ± 1.99	0.68
subrenal tumor growth [mg]	20.09 ± 8.03	21.62 ± 8.60	0.61

Table 2. Mean percentage of body weight loss and mean subrenal tumor growth (± SD) of rats having CO_2 pneumoperitoneum and air pneumoperitoneum (experiment II).

Discussion

Minimally invasive surgery has become rapidly popular because of its favorable postoperative course. Reduced postoperative pain, early mobilization and short hospital stay appear to result from a complex of factors[13]. Possibly, the most important factor is reduced surgical trauma due to the use of minimal incisions and minimally traumatic operative techniques[14]. This assumption has been validated by clinical studies showing lower levels of interleukin-6, which reflects tissue trauma, after laparoscopic surgery compared to open surgery[15,16]. Inflammatory response after laparoscopic surgery appears also less. In a comparative study on C-reactive protein levels after laparoscopic and open cholecystectomy lower levels of C-reactive protein were found after laparoscopic surgery[17]. Oncologically, laparoscopic surgery seems advantageous as shown in experimental studies in rats that showed less peritoneal tumor take and tumor growth after laparoscopic surgery[6,7]. Decreased extraperitoneal tumor growth after laparoscopic surgery was also reported by Allendorf et al. who found in a murine model significantly less subcutaneous tumour growth after insufflating the abdominal cavity as compared to laparotomy[18].
In spite of apparent advantages of laparoscopic surgery, concern exists about the dismal consequences of intraperitoneal insufflation of CO_2 [3]. Tumor recurrences in the abdominal wall after laparoscopic resections for cancer have been reported by

various authors[4]. Although the pathogenesis of port site metastases has not been completely resolved, insufflation of gas has been suggested to be an important factor. One hypothesis is that cancer cells concentrate at port sites due to leakage of aerosolized cancer cells which has been described as "chimney effect" by Kazemier et al[19]. A possible mechanism to reduce the incidence of abdominal wall metastases after laparoscopic surgery is to use gasless laparoscopy to prevent spreading of tumor cells by aerosolization and turbulence[20]. This assumption was confirmed in an experimental study in rats showing absence of port site metastases after after gasless laparoscopy[21].

In this study we attempted to assess systemic effects of different laparoscopic techniques by scoring tumor growth underneath the renal capsule. Subrenal tumor growth was less after gasless surgery in comparison to CO_2 pneumoperitoneum. Since subrenal tumor growth after air and CO_2 insufflation was similar in this study, the increased intraperitoneal pressure may be the most important factor causing increased tumor growth.

Increased intraperitoneal pressure induces a variety of reactions. Eleftheriadis et al. showed in an experimental study that intestinal ischaemia, free oxygen radical production, and increased bacterial translocation occurred in rats having a pneumoperitoneum[9]. Increased intraperitoneal pressure is also associated with greater neuroendocrine changes and decreased preservation of renal function compared with gasless laparoscopy for cholecystectomy[22]. Increased intraperitoneal pressure also causes decreased blood flow in parietal and visceral peritoneum which renders it susceptible to tumor growth[23]. Wu and Mustoe showed in an experimental study in rabbits that growth factors are more prevalent at ischemic sites promoting tumor growth[24]. Since growth factors have been shown to increase tumor growth in vitro, ischemia can be associated with tumor growth stimulation[25].

Air was used as an alternative to CO_2 in this study. Differences of tumor growth between air and CO_2 groups were not found. Watson et al demonstrated that phagocytotic activity of macrophages was less attenuated by intraperitoneal insufflation of CO_2 than intraperitoneal insufflation of air or laparotomy[26]. On the contrary, Jacobi et al. found more peritoneal tumor growth after air insufflation than after CO_2 insufflation in rats. In vitro studies showed similar stimulation of tumor growth by air and CO_2 in comparison to controls[27]. Therefore, it remains unclear if either air or CO_2 is preferable as an insufflating gas in laparoscopic surgery for malignancy.

Carbon dioxide insufflation also causes profound hemodynamic and respiratory changes[28]. Several studies showed that CO_2 insufflation in laparoscopic surgery causes hypercapnia and fatal complications such as gas embolism, arrhytmia or cardiac arrest. These complications stimulated use of alternative exposure of the abdominal cavity such as insufflating with air or mechanical elevation of the abdominal wall (gasless laparoscopy)[8,29]. McDermott showed that CO_2 insufflation unlike gasless laparoscopy led to a fall in partial blood oxygen pressures and absorption of CO_2 resulting in hypercapnia, acidosis, and consequent hyperdynamic circulation[30]. Davidson et al. showed that gasless laparoscopy may provide a safer alternative method of exposure for minimally invasive surgery in patients with pre-existing pulmonary or cardiac dysfunction than CO_2 insufflation[31]. Whether intraperitoneal CO_2 insufflation directly or indirectly via pH, hypercapnia or increased intraperitoneal pressure influences tumor growth remains unknown.

To evaluate the impact of CO_2 toxicity on metabolism, we compared postoperative body weight in rats having either CO_2 or air pneumoperitoneum. In this experiment, postoperative restoration of body weight was not different in the CO_2 and air group. To eliminate possible metabolic stress due to elevated intraperitoneal pressure, postoperative bodyweight was also assessed in rats having either CO_2 pneumoperitoneum or gasless surgery. Differences were also not found in this

experiment. Apparently, the negative hemodynamic, respiratory and hormonal effects of CO_2 insufflation did not affect the rate of postoperative recovery of body weight. However, it should be noted that body weight is a parameter for the entire complex of metabolic processes. Extrapolation of this observation to the clinical situation should be done with care because the duration of exposure of the peritoneal cavity to gas insufflation in this study was relatively short compared to clinical practice. Furthermore, the postoperative bodyweight observation period in this experiment was very short, only five days.

Although variables such as intraperitoneal pressure and operative time differed in this study from daily clinical practice, gasless laparoscopic bowel resection was associated with less tumor growth underneath the renal capsule as compared with CO_2 and air insufflation. Therefore, feasibility of application of gasless laparoscopy deserves further evaluation. The pathophysiological effects of intraperitoneal insufflation with either CO_2 or air in abdominal cancer are unclear and merit extensive study.

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Laparoscopic versus Conventional Bowel Resection in the Rat: Earlier Restoration of Serum Insulin-like Growth Factor 1 Levels

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Abstract

Introduction: Controversy exists on the role of laparoscopic surgery in treatment of colorectal disease. To assess the metabolic consequences of laparoscopic and open bowel surgery, we studied serum levels of insulin-like growth factor 1 (IGF-1), an anabolic and mitogeneic peptide, in rats.

Materials and methods: In experiment 1, serum IGF-1 levels of ten rats having laparoscopic small bowel resections (group I) and ten rats having conventional small bowel resections (group II) were determined before surgery and on day 1, 2 and 7. In experiment 2, five rats had CO_2 pneumoperitoneum (group III), five rats had laparotomy (group IV) and five rats had anaesthesia only (group V). Differences in IGF-1 levels were tested with analysis of covariance.

Results: In experiment 1, pre-operative IGF-1 levels were similar in group I and II; 87.9±6.1 nmol/L versus 90.5±8.1 nmol/L. One day after surgery IGF-1 was 54.6±10.5 in group I versus 41.6±8.3 in group II ($p=0.006$). Two days after surgery IGF-1 was 79.4±9.2 in group I versus 59.0±10.5 in group II ($p<0.001$). Seven days after both types of surgery, IGF-1 levels had almost returned to normal levels. In experiment 2, no significant differences were found between rats having either CO_2 pneumoperitoneum (group III) or rats having laparotomy only (group IV). Rats which had anaesthesia only, showed a significant decrease in IGF-1 levels between day 0 and 1 ($p<0.018$)

Conclusions: This study indicates that laparoscopic bowel surgery is associated with improved postoperative anabolic state (i.e. less catabolism) than conventional surgery, which underlines the potential benefit of laparoscopy in bowel surgery.

Keywords: insulin-like growth factor 1, laparoscopy, bowel, cancer.

Introduction

Minimally invasive surgery appears associated with less post-operative pain, shorter hospital stay and earlier return to normal activity than conventional surgery[1,2,3]. As a consequence of the advantages associated with minimally invasive surgery, laparoscopic techniques have also been employed in colorectal surgery. Although almost all colorectal procedures can be performed laparoscopically, laparoscopic colorectal surgery has not developed at the same rate as other laparoscopic procedures. Several factors have impeded widespread application of laparoscopic colorectal surgery. Advanced laparoscopic skills, costly and technically complex disposable instruments and long operative times are predicaments of laparoscopic colorectal surgery. In addition, concern about the radicality of laparoscopic resection and reports of port site metastases have held back the majority of surgeons to remove colorectal cancers laparoscopically[4,5,6].

Experimental studies are necessary to support the suggested advantages of the laparoscopic approach to colorectal surgery. Laparoscopic surgery is supposed to be associated with less disturbance of immunological and endocrinological functions. An earlier experimental study showed less loss of bodyweight post-operatively following laparoscopic surgery[7]. The bodyweight is supposed to reflect anabolic state. Another representative parameter of anabolism is insulin-like growth factor 1 (IGF-1). The insulin-like growth factor family of peptides, binding proteins, and receptors are important for normal human growth and development, and are involved in specialized functions of most physiologic systems. Most members of the IGF system are expressed by different cancer cells and may play an important role in the propagation of these malignant cells. New therapies aimed at modulating various components of the IGF system could affect the progression and metastasis of cancer[8,9].

Earlier experimental studies showed that peritoneal tumor take and tumor growth were significantly less after laparoscopic surgery[7,10]. Also tumor growth in the subrenal

capsule assay, which is a more precise, quantitative method to measure tumor growth showed less tumor growth after laparoscopic surgery[11]. Several other experimental studies have shown that surgery may facilitate tumor growth[12]. In these studies, the extent of the operative trauma appeared to be related to the degree of tumor growth stimulation. The operative trauma associated with laparoscopic surgery is considered to be less than that of conventional surgery[13]. The objective of this study is to assess metabolic sequelae of laparoscopic and conventional surgery, studying serum IGF-1 levels in rats.

Materials and methods

Animals

Male rats of the inbred WAG strain, weighing 200-300 g and aged 4-5 months were obtained from Harlan-CPB, Austerlitz, The Netherlands. Rats were bred under specific pathogen-free conditions. The animals were kept under standard laboratory conditions (Temperature 20-24°C, relative humidity 50-60%, 12 hours light/12 hours dark) and were fed a standard laboratory diet (Hope Farms, Woerden, the Netherlands) with free access to water and food before and after surgery. The protocols were approved by the Committee on Animal Research of the Erasmus University, Rotterdam, The Netherlands.

Operative Procedures

After being anesthetized with Atropine 0.05 mg/kg (Centrafarm), Domitor 0.25 mg/kg i.m. (Smithkline Beecham, Zoetermeer, The Netherlands), Ketalin 40 mg/kg i.p. (Apharmo b.b., Arnhem, The Netherlands), the abdomen of the animals was shaved. The rat was secured to the operating table with adhesive tape in a supine position and the abdomen was cleaned with 70% alcohol and dried with gauze.

The laparoscope, camera and attached cables were held at the desired angle by a

flexible arm. The surgeon was sitting at one end of the operating table facing the video monitor. The laparoscopic equipment and instruments (shortened to a length of 130 mm and with a diameter of 2 mm) were provided by Karl Storz Endoscopes and Duffner. The instruments and laparoscope were cleaned with 70% alcohol before and after surgery.

In experiment 1, rats undergoing a minimally invasive small bowel resection (group I) had a 5 mm skin incision in the midline of the abdomen at two-third between the xiphoid process and the pubis. A 5 mm laparoscopic sheath with insufflation side port was introduced followed by introduction of a 4 mm arthroscope. The pneumoperitoneum was created with CO_2 to a maximum pressure of 6 mm Hg. One operating port was placed in the right, and another in the left upper quadrant of the abdomen under direct vision. The operating ports consisted of shortened venous catheters with side port/homeostasis valve catheter (Arrow, Reading, Pennsylvania). The use of this operating port facilitated the insertion and withdrawal of instruments. The valve prevented CO_2 leakage through the port. After laparoscopic mobilization, a segment ileum of 8 cm in length was extracted and a small bowel resection (length 4 cm of the ileum) was performed. The anastomosis was performed extra-corporeally with a running suture using 7-0 Perma-hand Seide, Ethicon. The ports in the abdomen were closed with one suture, in one layer including muscle and skin (2-0 NC-Silk, B.Braun Melsungen AG).

In the conventional small bowel resection group II, a 5 cm abdominal skin incision was made in the midline of the abdomen. During operation, the viscera were removed from the abdominal cavity, put in phosphate buffered solution (PBS) wettened gauze and left extra-abdominally for approximately 20 minutes during which time a small bowel resection of 4 cm length took place. The anastomosis was made as mentioned above, the viscera were returned to the abdominal cavity and the abdomen was closed in one layer.

In experiment 2, rats had either a CO_2 pneumoperitoneum for 20 minutes without a small bowel resection (group IV) or a midline laparotomy without small bowel

resection (group IV). Group V had anaesthesia only during 45 minutes.
The operative time varied between 30 and 45 minutes in all procedures.

Experiment 1

On day one, twenty rats were randomized to two groups. Ten rats (Group I) underwent a laparoscopic small bowel resection and were compared with ten rats which had an conventional small bowel resection (Group II). Pre-operatively, after 1, 2 and 7 days, IGF-1 levels were measured.

Experiment 2

On day one, fifteen rats were randomized to three groups. Five rats underwent a CO_2 pneumoperitoneum (Group III), 5 rats had a laparotomy (Group IV) and 5 rats received anaesthesia only (Group V). Pre-operatively, after 1, 2 and 7 days, IGF-1 levels were measured.

Measurement of IGF-1

Serum IGF-1 levels were measured by radio immuno assay (RIA) with a kit from Medgenix (Belgium) and indicated in nmol/L. Intra-assay variation was maximally 6.1 % and inter-assay variation was maximally 9.9 %.

Statistics

The mean and the standard deviation (SD) of the collected data were calculated. To test for significant differences, analysis of covariance was used to compare IGF-1 levels between the groups[14]. To assess the presence of a normal distribution of the data, a histogram was made. All results proved to be normally distributed. Results were considered statistically significant at $p<0.05$.

Results

Experiment 1

Pre-operative IGF-1 levels were similar in group I and II; 87.9±6.1 nmol/L versus 90.5±8.1 nmol/L. One day after surgery IGF-1 was 54.6±10.5 nmol/L in group I versus 41.6±8.3 nmol/L in group II (p=0.005). Two days after surgery IGF-1 in group I was 79.4±9.2 nmol/L versus 59.0±10.5 nmol/L in group II (p<0.001). Seven days after both types of surgery IGF-1 levels had almost returned to normal, 86.0±5.7 nmol/L in group I versus 81.0±5.1 nmol/L in group II (Table 1, Figure 1).

Groups IGF-1	I n=10	II n=10	p-value IvsII
day 0	87.9±6.1	90.5±8.1	ns
day 1	54.6±10.5	41.6±8.3	0.005
day 2	79.4±9.2	59.0±10.5	<0.001
day 7	86.0±5.7	81.0±5.1	ns

Table 1. Mean levels and standard deviation of IGF-1 serum levels of rats having laparoscopic small bowel resection (group I), conventional small bowel resection (group II). The p-value represents differences between group I and II.

Experiment 2

Pre-operative IGF-1 levels were similar in group III, IV and V. One day after surgery IGF-1 was significantly lower in group III and IV as compared to pre-operative IGF-1 levels. Two days after laparotomy, CO_2 insufflation and anaesthesia IGF-1 levels had returned to normal levels. Seven days after each procedure IGF-1 levels were still normal (Tables 2 and 3, Figure 1).

Groups IGF-1	III n=5	IV n=5	V n=5	p-value III-IV
day 0	111.1±4.9	105.1±5.9	117.5±10.5	ns
day 1	88.9±3.8	87.7±11.7	103.0±9.3	ns
day 2	114.7±10.3	111.9±3.6	123.9±5.3	ns
day 7	100.4±5.4	104.4±5.7	106.8±6.9	ns

Table 2. Mean levels and standard deviation of IGF-1 serum levels of rats having CO_2 insufflation (group III), laparotomy (group IV) and anaesthesia only (group V). The p-value represents differences between group III and IV.

day Group	day 0-1 p-value	day 1-2 p-value	day 2-7 p-value
I	<0.001	0.018	ns
II	<0.001	<0.001	0.008
III	<0.001	ns	0.047
IV	0.045	ns	ns
V	0.018	ns	0.012

Table 3. P-values of differences within groups I, group II, group III, group IV and V comparing IGF-1 levels on day 0, 1, 2 and 7.

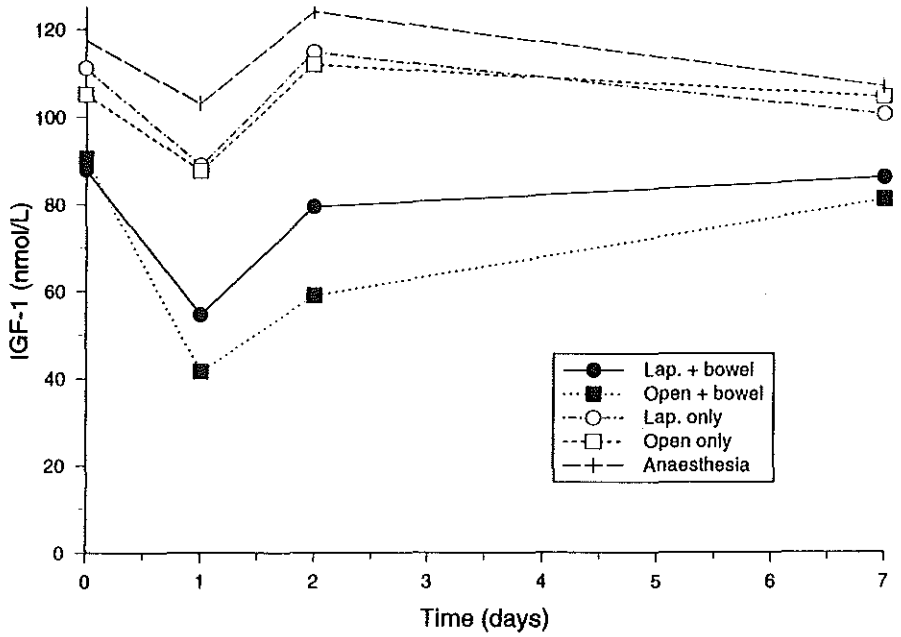

Figure 1. IGF-1 levels (nmol/L) on day 0, 1, 2 and 7 of each group.

Discussion

IGF-1 is structurally resembling insulin and has some similar effects. IGF-1 is transported and delivered to cell membrane receptors by different binding proteins. IGF-1 increases glucose and amino acid uptake and stimulates synthesis of protein and RNA, and in some cell types it promotes DNA synthesis, cell differentiation and prevents apoptosis[15,16]. A fall of serum IGF-1 levels following surgical trauma is thought to be caused by an increased efflux of IGF-1 from the blood to the peripheral tissues[17]. IGF-1 migrates to the extravascular tissues to promote metabolism, growth and regeneration. Therefore, decrease of serum IGF-1 levels can be regarded as a parameter of the extent of surgical trauma. In the present study, the fall of serum IGF-1 levels after laparoscopic small bowel resection was less than after conventional surgery

suggesting reduced surgical trauma. The decreased metabolic stress after laparoscopic surgery was shown in clinical studies of hormonal changes after laparoscopic procedures. Glaser et al. found higher serum levels of epinephrine and norepinephrine after conventional cholecystectomy than after open cholecystectomy[12]. Serum glucose levels and serum levels of C-reactive protein (CRP) proved to be lower after laparoscopic cholecystectomy as compared to conventional cholecystectomy[18]. Since rats are relatively inexpensive and detailed knowledge on their physiology is available, we have used rats to study the metabolic and oncological effects of laparoscopic surgery[19]. In a study assessing the post-operative body weight of rats after either laparoscopic or conventional small bowel resection, the rats which had laparoscopic surgery regained their pre-operative body weight significantly earlier than rats which had conventional surgery[8]. In the present study IGF-1 levels were less affected by laparoscopic small bowel resection than open small bowel resection. An interesting phenomenon was observed in this study when comparing IGF-1 levels in rats after after small bowel resection with rats after sham procedures. In the resection group, IGF-1 levels recovered more rapidly after laparoscopy. However, no differences were found between laparoscopy and laparotomy after sham procedures. Apparantly open bowel resection causes significant additional metabolic stress compared to laparoscopic small bowel resection.

The bodyweight is supposed to reflect anabolic state. In this study laparoscopic surgery in rats seems to be associated with less metabolic disturbances. In experiment 2, all groups showed higher IGF-1 levels at day 2 as compared to day 7. This phenomenon may reflect a compensation mechanism of the body reacting to a low IGF-1 level by IGF-1 production. This phenomenon was probably not seen in experiment 1 because IGF-1 was still consumed at the time of measurement (day 2) because these rats were exposed to greater trauma by the small bowel resection.

Clinical evidence of a correlation between metabolism and tumor growth has never been documented. However, growth stimulation of osteosarcoma cells by IGF-1 has been observed in vitro[8,9]. Other growth factors such as growth hormone and Epidermal Growth Factor have also been shown to increase tumor growth in vitro[20,21,22].

Our hypothesis is that the supposed tumor stimulating effect of IGF-1 is twofold. First, IGF-1 promotes DNA, RNA and protein synthesis both in normal and cancerous cells. Second, IGF-1 inhibits apoptosis, programmed cell death, which allows abnormally differentiated cells to proliferate. Higher IGF-1 levels in the tissue of rats which had a conventional bowel resection may result in stimulation of DNA synthesis, stimulation of cell growth and prevention of apoptosis. Therefore, the low serum IGF-1 levels (high tissue IGF-1 levels) and increased tumor growth, which was observed in earlier studies after conventional surgery could be causally related. Tumor take, as a parameter for growth of post-operative intraperitoneally implanted tumor cells, was assessed in an experimental study comparing rats having either laparoscopic or conventional small bowel resection. Significantly less tumor take was found in the laparoscopic group[8]. The subrenal capsule assay allows objective determination of tumor growth because the subcapsular tumor can be dissected easily and weighed accurately[13]. In an earlier study, tumor growth underneath the renal capsule was significantly greater in rats having conventional surgery than in rats having laparoscopic surgery or anesthesia only. Tumor growth in rats after laparoscopic small bowel resection was similar to that of rats which had anesthesia only. Apparently, tumor growth is proportional to the extent of surgical trauma. This was also shown by Eggermont et al., who found that peritoneal tumor growth was enhanced by laparotomy[11]. The finding of decreased tumor growth after laparoscopic surgery is in accordance with the report by Allendorf et al. who also found significantly less subcutaneous tumor growth after insufflating the abdominal cavity without any operation in a murine model as compared to laparotomy[23].

In summary, laparoscopic surgery seems associated with less attenuation of anabolism (i.e. lower catabolism) than conventional surgery. This may influence the potential value of the use of laparoscopic techniques in the treatment of malignant disease. However, further experimental studies and prospective randomized clinical trials are mandatory to establish the definitive role of laparoscopy in malignant disease.

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9

General Discussion

Introduction

Minimally invasive surgery has been shown to be associated with less post-operative pain, shorter hospital stay, earlier postoperative recovery and faster return to normal activity than conventional surgery[1,2,3]. Decreased metabolic stress after laparoscopic surgery was shown in clinical studies on hormonal changes after laparoscopic procedures. Glaser et al. found higher serum levels of epinephrine and norepinephrine after conventional cholecystectomy than after open cholecystectomy[4]. Serum glucose levels and serum levels of C-reactive protein (CRP) proved to be lower after laparoscopic cholecystectomy as compared to conventional cholecystectomy[5]. Reduced catabolism after laparoscopy was also shown by Glerup et al. who demonstrated that urea synthesis, as estimated by functional hepatic nitrogen clearance was increased after conventional cholecystectomy, but not after laparoscopic cholecystectomy[6]. Karayiannakis et al. showed that also neuroendocrine and inflammatory changes following laparoscopic cholecystectomy were reduced compared with conventional cholecystectomy[7].

Based on diminished metabolic stress of laparoscopic surgery, the hypothesis has been put forward that laparoscopic surgery would be associated with improved oncological prognosis. Tissue trauma is less in laparoscopic surgery compared to conventional surgery due to the use of minimal incisions, meticulous dissection facilitated by image magnification and the absolute necessity to perform bloodless surgery. The relationship between degree of tissue trauma and cancer has already been shown by Eggermont et al.. who have shown that tumor growth was proportional to the extent of the size of the laparotomy. Therefore, laparoscopic surgery appears beneficial in cancer. Possible metabolic and oncological benefits of laparoscopic surgery in comparison to conventional surgery have to be investigated.

However, in recent years, several reports of trocar site recurrence after laparoscopic oncological procedures have been published[8,9,10,11]. These reports have caused major turmoil because abdominal wall metastases had been rarely reported after conventional laparoscopic colorectal resection. Before oncologic laparoscopic procedures can be performed worldwide many questions need to be answered. The impact of different gases, use of gasless techniques, gas leakage and ischemia at trocar sites on tumor growth needs elucidation. Since it will take years to evaluate these aspects of laparoscopic techniques in randomized clinical trials, experimental animal studies can help to clarify these questions.

Animal Model

Large animals, such as pigs and dogs, are the preferred model for laparoscopic training[12]. However, these animals are expensive, and detailed knowledge of some aspects of their physiology is limited. Therefore, in this study a laparoscopic rat model, first described by Berguer et al., was used to investigate the metabolic and oncologic consequences of laparoscopic surgery[13]. We developed our own instruments with a diameter of 2 mm and operating table with an overhanging flexible arm to mount the laparoscope. Initially we had some problems with the shortened human trocars we used but with the use of alternative operating ports consisting of a shortened venous catheter this problem was solved. A pneumoperitoneum with a maximum pressure of 6 mm Hg proved to be optimal in this model. Serious anesthetic problems were seen if higher pressures were used. Anesthesia was first achieved by continuous ether inhalation. Since this kind of anesthesia may cause immunological disturbances and because ether is difficult to administer at a constant rate resulting in peroperative mortality, we started using systemic anesthetics.

One would expect minimally invasive surgery in small animals such as rats technically difficult. However, due to the magnification of the operative field, the surgeon gets the impression to be operating in a normal-sized space. Essential for laparoscopic surgery

in rats is availability of high quality instruments of 2 mm diameter. After some experience, intracorporeal suturing can be performed in rats. We started performing Nissen fundoplication followed by laparoscopic assisted small bowel resection. The bowel resection was used as a model in the oncological and metabolic experiments because it resembles clinical bowel surgery. Another important factor was that laparoscopic assisted small bowel resection was technically relatively simple and therefore not subject to variations in surgical performance. Laparoscopic assisted small bowel resection was hardly ever complicated by anastomosis leakage or persisted ileus. With growing experience, technically more difficult laparoscopic procedures could be performed in the rat. Laparoscopic splenectomy and nefrectomy proved technically feasible and might provide new localized tumor models to investigate oncological effects of laparoscopic surgery[14]. Furthermore adrenalectomy and rectum pull through procedure according to Swenson proved to be technically feasible in a rat model[15].

Metabolism

To assess the metabolic consequences of laparoscopic and open bowel surgery, we studied postoperative body weight loss and serum levels of insulin-like growth factor 1 (IGF-1) in rats. IGF-1 is an anabolic and mitogeneic peptide and migrates to the extravascular tissues to promote metabolism, growth and regeneration. IGFs are an integral part of the cytokines network. IGF-1 concentrations in serum are greatly stabilized by IGFBP-3 (binding protein), which serves as the major circulating carrier of IGF-1. Thus the steady state IGF-1 concentration will depend on the IGFBP-3 concentration. IGFBP-3 increases the IGF-1 half-life time in serum from 10 minutes to 10-15 hours. Therefore, IGF is an ideal molecule to measure because there is a relatively long half-life time and concentrations hardly fluctuate during the day[16]. A fall of serum IGF-1 levels following surgical trauma is thought to be caused by an increased efflux of IGF-1 from the blood to the peripheral tissues[17]. Therefore, decrease

of serum IGF-1 levels can be regarded as a parameter of the extent of surgical trauma. In our study, the fall of serum IGF-1 levels after laparoscopic small bowel resection was less than after conventional surgery suggesting reduced surgical trauma[18]. An interesting phenomenon was observed in this study when comparing IGF-1 levels in rats after small bowel resection with rats after sham procedures. In the resection group, IGF-1 levels recovered more rapidly after laparoscopy. However, no differences were found between laparoscopy and laparotomy after sham procedures. Apparently open bowel resection causes significant additional metabolic stress compared to laparoscopic small bowel resection.

The bodyweight is supposed to reflect an anabolic state. In our study laparoscopic surgery in rats seemed to be associated with less metabolic disturbances. In another study assessing the post-operative body weight of rats after either laparoscopic or conventional small bowel resection, rats that had laparoscopic surgery also regained their pre-operative body weight significantly earlier than rats which had conventional surgery[19]. Recently Davies et al. studied postoperative ileus after conventional laparoscopic assisted and totally laparoscopic bowel resection in a canine model. Laparoscopic resection techniques resulted in a more rapid return to normal gastrointestinal transit compared with open colectomy[20].

Relevance of tumor models

The optimal tumor model would be a localized tumor which could be removed laparoscopically and showed the same pattern of metastases as human colorectal cancer. Such a tumor model is still not available. In addition, other models were used to imitate different aspects of tumor behaviour during and after laparoscopic and conventional surgery.

In the *cell-seeding model* tumor cells were injected intraperitoneally directly after surgery (tumor take) and two days before each technical procedure (tumor growth). Studies on tumor biology after either open and laparoscopic surgery have shown less intraperitoneal tumor take and less intraperitoneal tumor growth after laparoscopic small bowel surgery as compared to open surgery in a rat model[19,21]. One of the problems with the cell suspension model is the assessment of either tumor take or growth. In these studies intraperitoneal tumor growth was scored semiquantitatively at different intra abdominal sites using the Steller-index to score intraperitoneal tumor load. Steller showed that similar results were obtained when visual scores (peritoneal cancer index), tumor mass and 125IUdR tumor uptake were assessed. Steller proved in his thesis that either of these three assessments as mentioned above can be employed for quantifying intraperitoneal tumor load, since no significant differences between the methods were found[22]. Therefore we chose the most simple method, visual assessment. The cell seeding model mimics the clinical situation of Dukes' D colon cancer which limits the applicability of the cell suspension model to daily clinical practice.

In the *solid tumor model*, a lump of tumor cells was placed intraperitoneally after different types of surgery and thereafter removed through either a trocar port or midline laparotomy. In this model, tumor cells might disperse unevenly within the peritoneal cavity due to traumatic extraction of the solid lump. Therefore the *subrenal capsule assay* (SRC) was developed which allows objective determination of tumor growth because the subcapsular tumor can be easily dissected and weighed accurately[23]. Disadvantage of this model is that a mini midline laparotomy has to take place 3 days before surgical intervention to place the tumor underneath the renal capsule. Possible immunological shifts can be overruled by this earlier surgical procedure. In our study, tumor growth underneath the renal capsule was significantly greater in rats having conventional surgery than in rats having laparoscopic surgery or anesthesia only[21]. Besides gasless laparoscopy was associated with less renal subcapsular tumor growth

than insufflation with CO_2[24]. The finding of decreased tumor growth after laparoscopic surgery is in accordance with the report by Allendorf et al.. who also found significantly less subcutaneous tumor growth after insufflating the abdominal cavity in a murine model as compared to laparotomy[25]. Mathew et al. demonstrated a five-fold increase in the incidence of metastases in the abdominal access wounds of rats undergoing laparoscopic surgery compared to conventional surgery, despite the advantage of reduced growth of the implanted 'primary' tumour[26]. Furthermore we developed a model to investigate the influence of local ischemia and gas leakage along on the development of abdominal wall metastases. Both ischemia and gas leakage were associated with more tumor growth at the trocar site.

Tumors

Colorectal cancer in humans is considered to be non or at best weakly immunogeneic. Evidence for this statement mainly originated from immunotherapeutic studies. Moertel et al. and Laurie et al. demonstrated some therapeutical effect when the immunomodulator levamisole was added to fluorouracil in patients operated for colorectal cancer[27,28]. Riethmüller et al. found that monoclonal antibodies can prolong recurrence in patients with colorectal cancer of Dukes' C stage[29].

Both experimental tumors used in this thesis are transplantable to WAG/Rij rats. CC531 colon adenocarcinoma is a weakly immunogenic tumor, sensitive to immunomodulation[30]. ROS-1 osteosarcoma originated spontaneously in the tibia of a rat and also sensitive to immunomodulation[31,32]. Both tumors grow in the same pattern. Tumor growth of intraperitoneally injected tumor cells could best be scored 4 weeks after injection. Pilot studies in which rats were sacrificed more than 6 weeks after intraperitoneal tumor cell injection showed tumor overgrowth. In these cases, it was impossible to score differences of tumor growth because of diffuse peritonitis carcinomatosa.

Abdominal wall metastases

Reports of abdominal wall metastases after laparoscopic colorectal surgery in cancer patients have caused major concern because abdominal wall metastases had been rarely reported after conventional laparoscopic colorectal resection. In large series Hughes and Reilly described abdominal wall metastases in the laparotomy incision, at the insertion site of a drainage tube and perineal recurrence, accounting for a rate of 0.6% and 1.5% respectively[33,34]. Vukasin et al. reported in the largest series until now that the incidence of wound recurrence after 480 laparoscopic colectomies was 1.1%[35]. In spite of these numbers, abdominal wall metastases withhold many colorectal surgeons to use laparoscopic techniques to treat colorectal cancer[36]. Since the pathogenesis of abdominal wall metastases is unresolved, basic scientific studies focusing on this topic are indicated. The development of abdominal wall metastases is probably multifactorial.

Tumor-Wound contact

Direct implantation of tumor cells at trocar sites can occur when a laparoscopic instrument has inadvertently grasped the tumor during the laparoscopic procedure. When this instrument is withdrawn through the trocar, tumor cells can detach from the trocar and adhere to the abdominal wall. Removal of the trocar at the end of the laparoscopic procedure can result in direct implantation of tumor cells at the trocar site. Extraction of the tumor through an small incision without protection of the abdominal wall appears to be the other variant of direct implantation of tumor cells[37]. Prevention of port site metastases due to direct tumor-wound contact can be provided accurate localization of the colorectal tumor intra-operatively and protection of the extraction site by foil or plastic bags[38,39]. In our study we found that significantly more tumor growth was found at the tumor extraction site as compared to non-extracting sites in laparoscopic oncological surgery[40].

Influence of pneumoperitoneum

One of the drawbacks of laparoscopic surgery is that intraperitoneal insufflation of CO_2 is required to create a working space. Carbon dioxide insufflation of the peritoneal cavity results in hypercarbia, acidosis, hemodynamic alterations and gut ischemia[41]. Consequences of elevated intra-abdominal pressure caused by insufflation will be discussed later. Though the interaction between CO_2 and macrophages on tumor defense mechanisms remain unsettled, several studies investigated direct effects of different insufflating gases on immune system and tumor growth. In the study by Watson et al., the release of cytokines by macrophages was less after CO_2 insufflation than after air insufflation or laparotomy[42]. Cytokines are essential mediators in the activation of lymphocytes that possibly play a role in killing tumor cells. However, phagocytotic activity of macrophages was less attenuated by CO_2 than air or laparotomy in the study by Watson. Jacobi et al. demonstrated that subcutaneous tumor growth was promoted by CO_2 insufflation as compared to helium and controls in a rat model.[43] Da Costa et al. demonstrated that endotoxin translocation is probably induced by factors in circulating air. In this study direct injection of endotoxin into the peritoneal cavity resulted in increases in intraperitoneal tumor growth similar to laparotomy[44]. In this thesis we found significantly less intraperitoneal tumor growth and less tumor growth underneath the renal capsule after laparoscopy as compared to laparotomy[21].

Effects of intraperitoneal insufflation can be caused by different types of insufflating gases or by elevated intraabdominal pressure. Eleftheriadis et al. showed in an experimental study that intestinal ischaemia, free oxygen radical production, and increased bacterial translocation occurred in rats having a pneumoperitoneum[45]. Increased intraperitoneal pressure causes decreased blood flow in parietal and visceral peritoneum which renders it susceptible to tumor growth[46]. A clinical study comparing different insufflation pressures for laparoscopic cholecystectomy showed significantly less hemodynamic changes and less postoperative pain was associated with low-

pressure insufflation[47]. No differences in tumor growth nor weight loss were found between rats which had insufflation with CO_2 and insufflation with air. On the other hand gasless laparoscopy was associated with less renal subcapsular tumor growth than insufflation with CO_2[24]. For this reason elevated intra-abdominal pressure might play an important negative role in laparoscopy for both benign and malignant disease and deserves further study.

In another study of ours, intraperitoneal free tumor cells were used to mimic the clinical situation of free viable tumor cells and to investigate the mechanisms of abdominal wall recurrence. In the cell seeding model port site metastases in the CO_2 group were significantly more frequent than in the gasless group[41]. In a hamster model Jones et al. demonstrated that pneumoperitoneum increased the implantation of free intra-abdominal cancer cells at wound sites and within the abdominal cavity[48]. Hewett et al. described intraperitoneal cell movement during abdominal CO_2 insufflation and laparoscopy in a porcine model[49]. Their results suggested that the movement of cells throughout the abdominal cavity during laparoscopy occurs via contaminated instruments. In vitro studies have shown that malignant cells adhere to laparoscopic instruments and laparoscopic ports during simulated laparoscopy[50]. In a pig model Allardyce et al. showed that tumor cell distribution is mainly caused by port manipulation and perhaps consequential leakage along trocar ports[51]. Studies on gasless laparoscopy indicated that frequent withdrawal of contaminated instruments contributes to trocar wound deposition. Whelan investigated both in vitro and in vivo if tumor cells can be aerosolized and showed that aerosols of tumor cells are not likely to form. Rapid desufflation could transport fluid loaded with tumor cells[52]. To prevent contact between ports and abdominal contents, all ports should be removed while the abdominal cavity is still insufflated. Another option is to wash all instruments routinely in a cytotoxic agent after withdrawal from a port. In addition, the trocar wounds can be impregnated with cytotoxic agents before and after introduction of trocars.

Gas leakage

It can be hypothesised that insufflation of CO_2 in the peritoneal cavity causes turbulence displacing tumor cells. At the port sites, concentration of tumor cells occurs as a result of the "chimney effect": leakage of CO_2 alongside trocars causes a high local gas flow at the trocar sites. The high flow of CO_2 may contain aerosols with viable tumor cells, which results in implantation of tumor cells at these sites[53]. Free intraperitoneal tumor cells occur frequently in digestive cancer as described by Juhl et al. who showed that 39 percent of patients operated for carcinoma of the stomach, colon, rectum and pancreas had free intraperitoneal malignant cells[54]. Spreading tumor cells, by manipulation or insufflation of gasses into the peritoneal cavity appears to affect survival of patients with malignant intraperitoneal tumors. Zirngibl et al. reported that intra-operative tumor cell spillage reduced the 5-year survival rate after resection of rectal cancer from 70 to 44 percent[55]. In this thesis, we showed gas leakage alongside trocars to be associated with more tumor growth at the site of the abdominal wound[56]. Passage of aerosols alongside trocars could be prevented by the use of gasless laparoscopic techniques and trocars with occlusive balloons.

Local factors

Local factors can influence the site and growth of metastases[57]. Eggermont et al. showed that surgery can augment tumor growth[58]. Enhanced tumor growth in scar tissue may be related to the presence of growth factors that are released following the process of tissue repair and inflammatory response[59,60]. Murthy et al. described the possibility of tumor cell entrapment in the clot formation of fresh wounds. Such clot formation could not only bind tumor cells but also offers nutrition and a barrier against host defense mechanisms[58]. Local ischaemia induced by trocar placement reduces local defense mechanisms rendering the ischaemic site vulnerable for tumour implantation and growth[61]. In the rat model described in this thesis, Tseng et al. demonstrated that trocar wounds that had been subjected to ischaemic trauma showed significantly more tumor growth[56].

Hematogenous or lymphogenous metastases

Another mechanism of development of abdominal wall metastases is either hematogenous or lymphogenous migration of tumor cells to the abdominal wall[62]. This seems unlikely because the blood and lymphatic vessels of the intra-abdominal organs lack an anatomical linkage with the abdominal wall. Moreover, some authors have reported port site metastases without any other evidence of metastases, suggestive of implantation metastasis[63]. Experimental studies have also shown that intravenously injected tumor cells may implant in intraperitoneal wounds but not occur in cutaneous wounds[64,65]. In clinical studies, colon cancer cells have been identified in the portal vein during surgery[66]. Experimental studies are necessary to assess the occurrence of free cancer cells in the blood stream and possible implantation at remote sites.

Other possible factors

Dexter et al. described that the duration of operation did not affect the risk of general complications after laparoscopic cholecystectomy[67]. In our experimental studies the operating time of all surgical procedures was in the same range. It seems interesting to investigate the consequences of prolonged operating time on metabolism and tumor growth. Intraperitoneal desiccation caused by insufflating gas causes a poorly oxygenated environment with release of several substances that stimulate regeneration. These factors may also stimulate tumor growth. Closing the abdominal wall in two layers, as in conventional surgery might also construct a mechanical barrier to the development of subcutaneous metastases. Perhaps less high insufflating pressures will be associated with a better metabolic and oncological prognosis. Wallace et al. demonstrated that insufflation pressure significantly affects the haemodynamic changes and postoperative pain associated with laparoscopic cholecystectomy[47]. Possibly routine administration of intraperitoneal chemotherapy after laparoscopic colectomy for cancer may improve prognosis[68].

Future prospects

Future research will revolve around creation of a localized tumor model. Such a model resembles the clinical situation more closely which will facilitate extrapolation of experimental results to humans. Extensive knowledge on direct effects of CO_2 on tumor cells is lacking and deserves study. In particular, pressure and time related effects need to be studied. Use of intraperitoneal and local chemotherapy merits further evaluation. Presence and possible consequences of intravascular tumor cells during surgery for cancer should be evaluated as well. While the results of these experimental studies are awaited, use of laparoscopic techniques in colorectal cancer should be limited to randomized clinical trials.

Conclusions

1. Laparoscopic surgery is associated with less intraperitoneal tumor take and less intraperitoneal tumor growth compared to conventional surgery. In addition, less tumor growth appears underneath the renal capsule when comparing laparoscopic surgery with conventional surgery.
2. Laparoscopic surgery correlates with less post-operative body weight loss than conventional surgery.

   Serum levels of insulin-like growth factor 1 show a significantly earlier recovery after laparoscopic surgery compared to conventional surgery.
3. Direct contact between solid tumor and the port site enhances local tumor growth. Gasless laparoscopy is related with less tumor growth underneath the renal capsule compared to CO_2 and air insufflation. Insufflation with CO_2 stimulates tumor growth at the trocar sites and is associated with more intraperitoneal tumor growth than gasless laparoscopy. Gas leakage alongside the trocar as well as local ischemia at the trocar site stimulate the development of abdominal wall metastases.

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Summary and Conclusions

Summary and Conclusions

Chapter 1 describes the history and development of laparoscopic techniques and outlines important landmarks that preceded present laparoscopic surgical practice. In 1977 Kurt Semm introduced the first CO_2 Pneu-Automatic insufflator, facilitating automatic monitoring of the intra-abdominal pressure, through which manual insufflation of the abdominal cavity was no longer required. A major improvement to laparoscopy was the attachment of a TV-camera to the laparoscope, which further enhanced laparoscopic surgery as well as surgical education. After Dubois reported the first large series of laparoscopic cholecystectomy in 1990, the application of laparoscopic techniques got widely accepted and other laparoscopic surgical areas followed rapidly.

Chapter 2 is the general introduction to this thesis. An overview is given of advantages and disadvantages of laparoscopic surgery in general and specifically in case of malignant disease. Laparoscopic colorectal resections are technically possible and seem to be associated with similar resection margins and numbers of harvested lymph nodes as open colorectal surgery. However, concern about reports of abdominal wall metastases after laparoscopic resections in case of malignant disease have held back the majority of surgeons to perform this type of surgery. Different experimental settings are discussed as well as their relevance for clinical practice. Chapter 2 concludes with a summary of the objectives of this thesis.

Chapter 3 presents a comparative study between rats that had either laparoscopic or open bowel surgery. Post-operative body weight loss was measured for seven days. This experimental model appears to correlate well with the clinical situation since reconvalescence, reflected by post-operative weight, after laparoscopic bowel resection

was significantly improved as compared to open surgery. Furthermore, differences in tumor take were investigated between rats that had laparoscopic and open bowel surgery and carbon dioxide pneumoperitoneum versus anesthesia alone. Directly after each surgical procedure, tumor cells were injected intraperitoneally. This study showed that laparoscopic bowel resection was associated with less peritoneal tumor take than open bowel surgery. The use of carbon dioxide pneumoperitoneum stimulated tumor take when compared to rats that had anesthesia alone. A 'Letter to the Editor' was published in response to chapter 3 in Surgical Endoscopy. It emphasized that in this tumor model tumor cells were not introduced in the abdominal cavity until after the surgical procedure was concluded. The authors warned not to draw conclusions of the potential beneficial effect of laparoscopic over open surgery until the mechanism of the occurrence of abdominal wall metastases has been solved.

Chapter 4 presents the results of a study comparing differences of growth of either pre-operatively intraperitoneally injected tumor cells or tumor underneath the renal capsule after either laparoscopic bowel resection, open bowel resection or anesthesia only. The purpose of this study was to evaluate the influence of different surgical procedures on established intraperitoneal tumor growth and tumor growth underneath the renal capsule.

Intraperitoneal tumor growth was scored semiquantitatively at several intraabdominal sites. Tumor growth underneath the renal capsule was excised and weighed.

Comparable to the outcome of the study in Chapter 3, less intraperitoneally tumor growth was found after laparoscopic surgery compared to open surgery. Rats that had anesthesia only showed less intraperitoneal tumor growth than rats that had laparoscopic bowel resection. In the subrenal capsule assay significantly more tumor growth was found comparing open bowel surgery with laparoscopic surgery and anesthesia only. No differences in tumor growth underneath the renal capsule were found between rats that had laparoscopic bowel resection and rats that received

anesthesia only. These results suggest strongly that reduced stimulation of tumor growth is inherent to minimally invasive surgery.

Chapter 5 describes a laparoscopic rat model to study peritoneal tumor growth and abdominal wall metastases after carbon dioxide pneumoperitoneum, gasless laparoscopy and laparotomy. Insufflation of the peritoneal cavity is considered to be a causative factor in the development of abdominal wall metastases after laparoscopic resection of malignant tumors. Important conclusions drawn from this chapter were (1) direct tumor wound contact enhanced local tumor growth and (2) CO_2 insufflation promoted tumor growth at the peritoneum and was associated with greater abdominal wall metastases than gasless laparoscopy.
'Letter to the Editor' presents three letters that are to be published as a reaction to chapter 5 in the Annals of Surgery.

Chapter 6 evaluates the influence of both local ischemia and gas leakage on the development of abdominal wall metastases at the trocar sites. The traumatized trocar wound appears a good medium for implantation and growth of tumor cells, because growth factors are abundantly present in traumatized tissue. Leakage along the trocar could result in high local flow containing free floating tumor cells. These hypotheses require the presence of free viable tumor cells in the peritoneal cavity and transportation of these tumor cells to the trocar wounds.
In the first experiment, rats had four trocars placed in the abdominal cavity. Two of the trocar wounds in each rat were crushed to induce ischemia. Thereafter, insufflation of the abdominal took place after injection of CC531 tumor cells. In the second experiment, each rat had both a 5 mm and a 10 mm incision in the abdominal wall; 5 mm trocars were inserted in both incisions. Again, CC531 tumor cells were injected intraperitoenally and thereafter insufflation with CO_2 took place. After 4 weeks, all rats were sacrificed and tumor deposits at trocar sites were weighed. In this study, both

local ischemia and gas leakage promoted tumor growth at trocar sites. To prevent local ischemia, an incision which is too small must be avoided. Possibly, the use of balloon trocars can prevent gas leakage and reduce local tissue trauma.

Chapter 7 evaluates body weight and tumor growth after laparoscopy with CO_2 , air, or gasless laparoscopy. CO_2 insufflation of the peritoneal cavity results in hypercarbia, acidosis, hemodynamic alterations and gut ischemia; conditions that can possibly negatively influence metabolism and oncology.
In this study post-operative body weight refected the overall recovery from different types of surgery. We attempted to assess systemic effects of different laparoscopic techniques on tumor behaviour by scoring tumor growth underneath the renal capsule. The subrenal capsule assay was used because it allows accurate assessment of tumor growth in contrast to peritoneal tumor models.
In this study, subrenal tumor growth was less after gasless surgery in comparison to CO_2 pneumoperitoneum. Since subrenal tumor growth after air and CO_2 insufflation was similar in this study, the increased intraperitoneal pressure may be an important factor causing increased tumor growth.

Chapter 8 elaborates more on the metabolic consequences of both laparoscopic and conventional surgery. To study post-operative recovery both body weight and serum levels of insulin-like growth factor 1 (IGF-1) were measured. The bodyweight is supposed to reflect anabolic state. Another representative parameter of anabolism is IGF-I. In this study, we found that laparoscopic surgery in rats was associated with both less post-operative weight loss and improved post-operative restoration of IGF-I levels in serum.
The Insulin-like Growth Factor family of peptides, binding proteins, and receptors are important for normal human growth and development. IGF-I promotes DNA, RNA and protein synthesis both in normal and cancerous cells. On the other hand, IGF-I inhibits

apoptosis, programmed cell death, which allows abnormally differentiated cells to proliferate. A fall of serum IGF-1 levels following surgery is thought to be caused by an increased efflux of IGF-1 from the blood to the peripheral tissues. Therefore, the low IGF-I levels and reduced tumor growth, which was observed in other studies, could be causally related.

Chapter 9 includes the general discussion of this thesis and conclusions. It is important that, whilst the results of both experimental and clinical studies are awaited, the use of laparoscopic techniques in patients with colorectal cancer should be limited to randomized clinical trials.

Conclusions

1. Laparoscopic surgery is associated with less intraperitoneal tumor take and intraperitoneal tumor growth than conventional surgery. In addition, less tumor growth appeared underneath the renal capsule when comparing laparoscopic with conventional surgery.
2. Laparoscopic surgery is followed by less post-operative body weight loss than conventional surgery. Serum levels of insulin-like growth factor 1 show a significantly earlier recovery after laparoscopic surgery compared to conventional surgery.
3. Direct contact between solid tumor and port site enhances local tumor growth.
4. Gas leakage alongside the trocar as well as local ischemia at the trocar site stimulate the development of abdominal wall metastases.
5. Gasless laparoscopy is related with less tumor growth underneath the renal capsule compared to CO_2 and air insufflation. Insufflation with CO_2 stimulates tumor growth at trocar sites and is associated with more intraperitoneal tumor growth than gasless laparoscopy.

Samenvatting en Conclusies

Samenvatting en Conclusies

Hoofdstuk 1 bevat een korte beschrijving van de belangrijkste ontwikkelingen in de laparoscopische chirurgie. In 1977 ontwikkelde Kurt Semm de eerste automatische CO_2 insufflator, waardoor het zicht gedurende voornamelijk gynaecologische laparoscopische ingrepen aanzienlijk werd verbeterd. Vanaf 1986 was het mogelijk door middel van een videocamera een operatie direct via een beeldscherm te volgen. Onderwijs in de laparoscopische technieken werd hierdoor aanzienlijk vereenvoudigd. In 1990 publiceerden zowel Dubois als Mouret een grote serie succesvolle laparoscopische cholecystectomieën, hetgeen een verbreding van het indicatiegebied voor laparoscopische ingrepen in de algemene chirurgie tot gevolg had.

Hoofdstuk 2, de algemene introductie van dit proefschrift, geeft een overzicht van de literatuur over de voor- en nadelen van minimaal invasieve chirurgie. Laparoscopische colorectale chirurgie is technisch mogelijk. Diverse onderzoeken tonen aan dat er bij laparoscopische colorectale chirurgie gelijke aantallen lymfeklieren worden gereseceerd en dat de resectie marges van gelijke grootte zijn. De literatuur echter verbindt één nadeel aan het laparoscopisch opereren van het colorectale carcinoom: mogelijk treden na laparoscopische resectie frequenter buikwand-metastasen op dan na conventionele chirurgie.
In dit hoofdstuk worden de in dit proefschrift gebruikte laparoscopische rattenmodellen beschreven, die werden ontwikkeld om de relatie tussen laparoscopie, kanker en metabolisme beter in kaart te brengen, alsmede hun relevantie voor de kliniek. Aan het eind van hoofdstuk 2 worden de doelstellingen van dit proefschrift uiteengezet.

Hoofdstuk 3 geeft de resultaten weer van de eerste studie, waarbij verschillen werden onderzocht in postoperatief gewichtsverlies en tumor-take tussen ratten die via

laparoscopie danwel laparotomie een open dunne darm resectie ondergingen. De ratten werden gedurende zeven dagen postoperatief gewogen. In deze studie bleken ratten na laparoscopische dunne darm resecties sneller in gewicht toe te nemen.
Tumor-take is een maat voor het enten en daarna uitgroeien van direct post-operatief intraperitoneaal geïnjecteerde tumor-cellen. In dit hoofstuk werd onderzocht of er verschillen in tumor-take waren na laparoscopische en open dunne darm resecties. Daarnaast werd onderzocht of er verschil bestond in tumor-take tussen ratten na CO_2 insufflatie en ratten die alleen anesthesie toegediend kregen. Direct na elke procedure werden de tumor-cellen intraperitoneaal ingespoten. Vier weken na deze ingreep vond obductie plaats en werd de intraperitoneale tumor-groei semi-kwantitatief bepaald. Aangetoond werd dat na laparoscopische dunne darm resectie significant minder tumor-take optrad dan na open dunne darm resectie. Het insuffleren van de peritoneaal holte met CO_2 bleek de intraperitoneale tumor-take te stimuleren in vergelijking met ratten die alleen anesthesie kregen toegediend.
De aan dit hoofdstuk toegevoegde 'Letter to the Editor' bevat een reactie op dit hoofdstuk, zoals gepubliceerd in Surgical Endoscopy.

Hoofdstuk 4 bespreekt de resultaten van een studie naar de uitgroei van pre-operatief intraperitoneaal geïnjecteerde tumor-cellen en onder het nierkapsel aangebrachte tumor-cellen. Onderzocht werd of er verschil in tumor-uitgroei was na laparoscopische darmresecties, na open darmresecties en na toediening van uitsluitend anesthesie. Intraperitoneale tumor-groei werd semi-kwantitatief gescoord en tumor-groei onder het nierkapsel werd na obductie gewogen. De uitkomsten van deze studie zijn vergelijkbaar met die van hoofdstuk 3; minder intraperitoneale tumor-groei werd geconstateerd na laparoscopische chirurgie in vergelijking tot open chirurgie. Ratten die alleen onder narcose waren gebracht vertoonden minder intraperitoneale tumor-groei dan ratten die een laparoscopische resectie ondergingen.
In het subrenal capsule assay werd significant meer tumor-groei geconstateerd na open

darm resectie in vergelijking tot zowel laparoscopische resectie, als uitsluitend anesthesie. Tumor-groei onder het nierkapsel bleek niet verschillend tussen laparoscopisch geopereerde ratten en ratten die alleen narcose hadden gehad. De resultaten van deze studie suggereren dat een relatie bestaat tussen verminderde tumor-groei stimulatie en minimaal invasieve chirurgie.

Hoofdstuk 5 beschrijft een rattenmodel waarin de invloed van laparotomie, CO_2 pneumoperitoneum en gasloze laparoscopie op de ontwikkeling van buikwand-metastasen en intraperitoneale tumor-groei wordt onderzocht. Verwacht werd dat mogelijk insufflatie van de peritoneale holte een belangrijke rol speelt bij de ontwikkeling van buikwand-metastasen na laparoscopische colorectale chirurgie voor maligniteiten. De belangrijkste conclusies die naar aanleiding van deze studie konden worden getrokken zijn: (1) direct contact tussen tumor en trocar-wond bevordert tumor-groei ter plaatse van de trocar opening en (2) CO_2 insufflatie veroorzaakt meer intraperitoneale tumor-groei en meer tumor-groei ter plaatse van de trocar openingen dan gasloze laparoscopie.
Aansluitend op dit hoofdstuk worden drie reacties op deze studie beschreven die zullen worden gepubliceerd in the Annals of Surgery ('Letters to the Editor').

Hoofdstuk 6 behandelt twee rattenmodellen. De invloed van zowel locale ischemie als de lekkage van gas langs de trocar op de ontwikkeling van buikwand-metastasen werd onderzocht. Het eerste model neemt in ogenschouw, dat groeifactoren mogelijk in hoge mate aanwezig zijn in de getraumatiseerde trocar-wond en dat deze ischemische plaats een goede voedingsbodem voor tumor-cellen zou kunnen zijn om uit te groeien. Het tweede model onderzoekt in hoeverre een pneumoperitoneum tumor-cellen zou kunnen opnemen in een aërosol en of verplaatsing van deze cellen naar een lekkende trocar, de plek van de minste weerstand, mogelijk is.
Zowel locale ischemie als gas-lekkage bleken tumor-groei ter plaatse van de trocar te

stimuleren. Het lijkt dan ook verstandig om locale ischemie te voorkomen door incisies niet te klein te maken en lekkage te voorkomen, bijvoorbeeld door het gebruik van ballon trocars.

Hoofdstuk 7 beschrijft een studie waarin onderzocht werd of er verschillen zijn in post-operatief gewichtsverlies en tumor-groei onder het nierkapsel na diverse laparoscopische technieken: laparoscopie met insufflatie van CO_2, van lucht, of gasloze laparoscopie. CO_2 insufflatie veroorzaakt hypercapnie, acidose, hemodynamische schommelingen en darm-ischemie. Verhoogde intraperitoneale druk (door lucht of CO_2) veroorzaakt produktie van vrije zuurstof radicalen en verhoogde bacteriële translocatie. Deze veranderingen kunnen mogelijk een negatieve weerslag hebben op het metabolisme en oncologische prognose.
In deze studie werd geen verschil geconstateerd in postoperatief herstel tussen de diverse laparoscopische technieken.
Wel werd minder tumor-groei onder het nierkapsel gesignaleerd na gasloze laparoscopie in vergelijking tot laparoscopie met een CO_2 pneumoperitoneum. Er werd geen verschil in tumor uitgroei gevonden tussen ratten die CO_2 laparoscopie ondergingen vergeleken met lucht laparoscopie. Uit deze studie kunnen wij concluderen dat verhoogde intraperitoneale druk mogelijk een negatieve rol speelt bij de acceleratie van tumor-groei.

Hoofdstuk 8 onderzoekt de invloed van laparoscopische en conventionele chirurgie op metabolisme. Als maat voor postoperatief herstel van metabolisme werden zowel postoperatief gewichtsverlies als insulin-like growth factor 1 (IGF-1) gemeten. Het lichaamsgewicht vormt evenals IGF-1 een maat van anabolisme. In deze studie werd aangetoond dat na laparoscopische chirurgie minder gewichtsverlies optrad dan na open chirurgie. Na laparoscopische chirurgie werd eveneens sneller herstel geconstateerd van de concentratie IGF-1 in het serum.

IGF gerelateerde stoffen zijn belangrijk voor normale groei en ontwikkeling. IGF-1 reguleert de synthese van DNA, RNA en eiwitten bij zowel normale als kanker-cellen. Daarnaast remt IGF-1 processen als apoptose en geprogrammeerde celdood, zodat slecht gedifferentieerde cellen zich kunnen vermenigvuldigen. Een lage IGF-1 concentratie in het serum duidt op een hoge concentratie IGF-1 in de weefsels, alsmede een groot verbruik aldaar. Het zou derhalve mogelijk kunnen zijn dat er een relatie bestaat tussen lage serum IGF-1 waarden en stimulering van tumor-groei.

Hoofdstuk 9 behelst de algemene discussie van dit proefschrift. Het geeft een overzicht van de resultaten van de in dit proefschrift beschreven studies en de mogelijke gevolgen van deze resultaten voor de kliniek. Het is belangrijk dat, zolang experimentele en klinische studies nog geen uitkomst hebben geboden ten aanzien van het mechanisme van de buikwand-metastasen, laparoscopische colorectale resecties voor maligniteiten slechts in trial verband plaatsvinden.

Conclusies

1. Laparoscopische chirurgie gaat gepaard met minder intraperitoneale tumor-take en tumor-groei, vergeleken met conventionele chirurgie. Eveneens wordt minder tumor-groei geconstateerd onder het nierkapsel na laparoscopische chirurgie in vergelijking tot conventionele chirurgie.
2. Na laparoscopische chirurgie volgt minder post-operatief gewichtsverlies dan na open chirurgie. De concentratie insulin-like growth factor 1 blijkt sneller terug op normaal waarde na laparoscopische chirurgie dan na open chirurgie.
3. Direct contact van tumor met (trocar)wond vergroot locale uitgroei van tumor in de buikwand.
4. Zowel locale ischemie als lekkage van gas langs de trocar stimuleren de vorming van buikwand-metastasen.

5. Na gasloze laparoscopie wordt significant minder tumor-groei in het subrenal capsule assay vastgesteld dan na laparoscopie met CO_2- of lucht-insufflatie. Daarnaast leidt CO_2 insufflatie vaker tot buikwand-metastasen en treedt er meer intraperitoneale tumor-groei op dan na gasloze laparoscopie.

Dankwoord

Dankwoord

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