PORT-SITE METASTASES IN LAPAROSCOPIC SURGERY
AN EXPERIMENTAL STUDY

Philippe Wittich

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CHAPTER 1

INTRODUCTION

INTRODUCTION

Endoscopic imaging and instrumentation have allowed revolutionary changes in surgery. For many decades, in spite of pivotal improvements in and around surgery, the surgeon's hands and relatively simple instruments were the key elements of operative procedures. Manipulating tissues by hands and instruments under direct vision involves traumatizing healthy tissues. Avoiding undue tissue trauma is the goal of endoscopic surgery.

Basics of endoscopy

Endoscopy covers a broad field of techniques to visualise inner parts of the body. It always involves a rigid or flexible scope composed of optical lenses and illumination of the inner cavity by a light source. Nowadays, all scopes can be connected to a TV-chip camera which transmits the image to a monitor, enabling participation of others than the surgeon in the operation. Endoscopic surgery requires creation of a space in the human body. Hereto, gas or liquid under pressure are used. Air in gastro- or colonoscopy, water in cysto- and arthroscopy and carbon dioxide in laparoscopy. Endoscopy can be conducted through natural orifices such as the mouth in gastroscopy, the anus in colonoscopy or urethral meatus in cystoscopy, or through an incision which is made in thoracoscopy, arthroscopy or laparoscopy. Laparoscopic surgery requires multiple small incisions to allow the introduction of endoscopic and laparoscopic instruments. To preserve gas or liquid pressure, special cannulas with valves (i.e. trocars or ports) are employed. To limit tissue damage and avoid infection, sterile and atraumatic products are used to create space. Carbon dioxide is a physiologic gas that can be absorbed rapidly in large amounts in blood without the risk of gas embolism. It is non explosive which allows use of diathermy. As carbon dioxide pneumoperitoneum causes hypercarbia and hypertension, alternatives were developed in the past decade. Inert gases such as helium were evaluated. Systems to create space by mechanically lifting or suspending the abdominal wall for laparoscopic procedures, have been developed. Still, carbon dioxide is considered the most safe and practical means to enable laparoscopic surgery and is therefore most widely used.

Laparoscopy in general surgery

Laparoscopy was started by internists and gynaecologists for diagnostic inspection at the beginning of the twentieth century[1,2,3]. From the 1930's, minor procedures such as taking diagnostic liver tissue samples, clipping ovarian tubes for sterilisation or adhesiolysis were performed laparoscopically[4]. In spite of many technical improvements in the second half of the last century, general surgeons did not show interest in laparoscopy for several decades. The first laparoscopic assisted appendectomy was described in 1977 by De Kok, but it would still take 20 years before laparoscopic appendectomy gained more general acceptance by surgeons[5]. Until the TV-chip camera was developed in 1983, the surgeon had to bend over to look through the scope and assistants were blinded to the procedure. In 1985, Mühe performed the first laparoscopic cholecystectomy with self-developed instruments[6]. Initially, there was limited interest in this new technique, but from 1987 laparoscopic resection of the gallbladder superseded the conventional approach in just a few years[7,8]. Patient recuperation and cosmesis were better and hospitalisation was significantly shorter in laparoscopically operated patients[6-8]. An important hypothesis regarding the benefit of laparoscopic surgery was that avoidance of a painful abdominal wound favoured fast mobilisation and normal respiration, thereby limiting hospitalisation and respiratory complications[9]. The procedure was technically more demanding and time consuming than an open approach, but the results convinced many that laparoscopic surgery should be developed further. Already in 1991, laparoscopic assisted colonic resections were described by Jacobs et al.[10] and this procedure was adopted by many worldwide. In the first half of the 1990's, laparoscopic technique spread rapidly to various procedures such as appendectomy, herniorrhaphy, colon resections, anti-reflux surgery, adrenalectomy and splenectomy.

Laparoscopic oncologic surgery

In laparoscopic colon surgery, attention was initially focused on feasibility of laparoscopic procedures and possible benefits for patients with both benign and malignant disorders. In curative oncologic surgery, however, disease free survival is the prime objective and duration of patient recuperation or length of scars are less important. Critics argued

that surgical oncologic principles such as radical resection, adequate margins and avoidance of tumor spill, were not met in laparoscopic surgery. Higher recurrence rates after laparoscopic resections were feared. At the same time, some case reports described wound metastases after laparoscopic cholecystectomy (in cases of preoperative unknown gallbladder cancer) and colon resections[11-14]. These trocar wound or port-site metastases were considered a new phenomenon induced by the laparoscopic technique. Initial reports suggested a high incidence of these abdominall wall recurrences. A decline of laparoscopic surgery in patients with curable colon cancer was the consequence. Performing laparoscopic colectomy for cancer was only tolerated within the frame of randomised clinical trials[15,16]. Registries and prospective or randomised trials were designed to gather data and assess quality of laparoscopic colon resections for malignancy[17-21]. Simultaneously, theories on trocar wound metastases and oncologic consequences of laparoscopy were developed and tested in the laboratory[22].

Laparoscopy differs from conventional surgery in several aspects. Laparoscopy is considered to induce less surgical trauma. As a consequence, catabolism is diminished and immune responses are better preserved, which results in less tumor growth[23,24]. Another difference is the increased technical difficulty of laparoscopic surgery compared to conventional surgery[25]. Loss of tactile senses and the fixed position of trocars which limits the degree of freedom of laparoscopic instruments render operative procedures more difficult. Another factor is insufflation. Insufflation of a gas at a certain pressure has introduced two phenomena: a gas flow in the abdomen and gaseous pressure on peritoneum and abdominal organs. Both can influence the dispersal and implantation of tumor cells[26,27]. Insufflation causes ischemia of the peritoneal lining of the abdominal cavity and it reduces flow in the portal and caval vein. Furthermore, hypothermia can occur when cold and dry gas is insufflated. Characteristics of the gas itself may influence chemical reactions and function of cells such as macrophages[28-30].

The objectives of this thesis are:

1 - to study the impact of intra-abdominal gas flow on development of port-site metastases

2 - to investigate the oncological consequences of different insufflation pressures and use of alternate gases

3 - to relate surgical techniques to tumor growth in the abdominal wall.

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CHAPTER 2

PORT-SITE RECURRENCES IN LAPAROSCOPIC SURGERY.

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Introduction

Tumor growths at port-sites after laparoscopic surgery for abdominal cancer have caused major concern among doctors and patients in the early 1990's[1]. Laparoscopic surgery was considered less traumatic for the patient, but appeared deceiving in malignancies in some patients. Many surgeons have abandoned the laparoscopic approach in patients with malignant tumors for fear of port-site recurrences. A wide variety of experimental studies have been instigated to unravel the pathogenesis of port-site recurrences in laparoscopic surgery. Are we facing a novel problem caused by a surgical technique that has been accepted to be superior to conventional open surgery too readily? Browsing through ancient medical literature revealed some interesting communications. In 1903, von Mikulicz published an article entitled 'Small contributions to the surgery of the intestinal tract'[2]. The Mikulicz procedure involved exteriorization of a colonic cancer through a small incision to allow extra corporal resection. The incentive of this procedure was to minimize fecal spill in the peritoneal cavity to prevent abdominal abscesses in an era devoid of antibiotics. More than half of the patients developed a cancerous recurrence in the abdominal wall within four years. Ninety years later, this phenomenon was encountered again at narrow extraction sites employed for laparoscopic removal of cancerous specimens[3]. In 1907 Charles Ryall stated in an article on 'Cancer infection and cancer recurrence: a danger to avoid in cancer operations' that "...cancer cells must have been liberated during manipulation of the growth and carried to the abdominal incisions by the instruments, suture, or surgeon's hands...."[4]. Almost one century later, the great vision of Ryall was confirmed in the laboratory by Hewett et al, who detected cancer cells on laparoscopic instruments after manipulation in rats with tumor call infested abdominal cavities[5]. The present literature on cancer recurrence at port-sites involves a large volume of case reports, small series and attempts to review all these individual cases. Thorough analysis of these histories can provide guidance in resolving the complex pathogenesis of port-site recurrences. In this chapter, port-site recurrences of gynecological malignancies, colon cancer, gallbladder cancer, urological malignancies and after diagnostic laparoscopy will be discussed subsequently.

Gynecological malignancies

Port-site recurrences after gynecological laparoscopy have been reported for all cancers of the female reproductive organs. However, laparoscopy for ovarian cancer is most commonly associated with port-site recurrences. In a review of 19 cases of metastases at the trocar insertion after laparoscopy for gynecological malignancy, 14 occurred after surgery for ovarian cancer. Curiously, 3 of these 14 ovarian tumors were borderline tumors[6]. Most of the procedures that were followed by port-site recurrences involved laparoscopic puncture or biopsy of ovarian tumors. The true incidence of port-site recurrences after laparoscopy for ovarian cancer is difficult to access. Other authors noted only one metastasis at a trocar site in 70 women with ovarian cancer, accounting for an incidence of 1.4%[7]. Reports of port-site recurrences after laparoscopy for cervical cancer are rare[8]. Only two reports exist of metastasis of cervical cancer in surgical scars after open surgery. One in the abdominal incision after radical hysterectomy for stage 1b squamous cell carcinoma of the cervix[9]. One case report has been published on port-site recurrences after laparoscopic surgery for endometrial cancer[6]. In this case, laparoscopic assisted vaginal hysterectomy had been done combined with laparoscopic pelvic and aortic lymphadenectomy for stage IIIc disease. Metastases occurred after an interval of 6 months at multiple port-sites and at the episiotomy scar that had been employed for extraction of the specimen. Relaparotomy for bowel obstruction revealed diffuse peritoneal carcinomatosis. Kadar showed in a retrospective review of 24 patients who had laparoscopic surgery for metastatic gynecological disease, port-site recurrences in 16 % of all patients[10]. Advanced stages of the disease clearly predispose to port-site recurrences.

Colorectal cancer

The first laparoscopic resections for colorectal cancer were performed in 1991[11]. The striking reduction of morbidity after laparoscopic removal of the gallbladder ignited great enthusiasm for laparoscopic colorectal surgery. However, in 1993 several alarming case reports on port-site recurrences after laparoscopic resection of colorectal cancer startled general surgeons and evoked great criticism among conventional surgeons about the ap-

plication of laparoscopic techniques in the treatment of colorectal cancer[12-14]. Particularly, reports of port-site recurrences after laparoscopic resection of Duke's A carcinomas caused major turbulence[15-17]. In one of these cases, a polypectomy was performed after colotomy[16]. At microscopy, this polyp contained a Duke's A carcinoma. Possibly, either inadvertent grasping of an occult tumor, that was not visible on the serosal side of the bowel, or tumor cell seeding through the lumen caused port-site recurrences in these Duke's A cases. Although port-site recurrences had been reported by the end of 1994 in less than 30 patients worldwide, laparoscopic treatment of colorectal malignancy became and remains very controversial. Many suggested that abdominal wall recurrence after colorectal cancer resection occurred exclusively after laparoscopy, while tumor recurrence at the abdominal wall after conventional resection of colorectal cancers has been suggested to occur rarely. Reilly reported in a study of 1171 patients who had open curative resections for colorectal cancers that the incidence of abdominal wall recurrence varied from 0.6 % to 0.9 % for either Duke's B or C stages[18]. These figures are in agreement with the 0.8 % incidence rate of abdominal wall recurrence that had been documented by Hughes et al. in 1983[19]. However, the method of detection appears to play an important role in determining the true incidence of abdominal wall recurrences. Welch and Donaldson discovered 16.6 % wound recurrence in 145 autopsies performed in patients with recurrent colorectal cancer[20]. Others encountered cancer recurrence in the abdominal wall of more than 3 % of patients who underwent a second look operation after a curative resection of colorectal cancer[21]. Two-thirds of these abdominal wall recurrences were at the level of the fascia and had not been found at physical examination. Extensive review of the literature until 1999 revealed 82 port-site recurrences after laparoscopic colorectal surgery (Table 1). Port-site recurrences occurred after all types of colorectal resections. In this overview, a predominance of laparoscopic right hemicolectomy appeared to be present. The tumor stages were known in 72 cases in our review and of these, 5 were classified as Duke's A, 11 as Duke's B, 42 as Duke's C and 14 as Duke's D. Disseminated disease at the time of diagnosis of the port-site recurrences was reported in 15 patients. All these patients had colorectal cancers staged as either Duke's C or Duke's D at the primary colorectal operation. Port-site recurrences were found at ports either used for instrumentation or the laparoscope and at extraction sites. The median interval until discovery of port-site recurren-

ces was 6 months. The earliest documentation of a port-site recurrence was 1 month after surgery, while the longest interval was 44 months. In 33 patients, the interval between surgery and the discovery of the port-site recurrence was recorded. Of interest is that only 5 (15 %) of these 33 patients developed port-site recurrences more than 1 year after the colorectal resection. The treatment and outcome of port-site recurrences after laparoscopic colorectal surgery has been reported in very few cases. Surgical inexperience appears an important etiologic factor for port-site recurrences considering the high incidences reported in early small series and the far lower rates in later reports on groups with more patients[13,22]. Therefore, it appears appropriate to only consider studies with more than 50 cases for assessment of the incidence of port-site recurrences after laparos-copic colorectal surgery. In 3547 patients, culled from series with more than 50 cases, port-site recurrences were recorded in 30 patients accounting for an incidence of 0.85 % (Table 2).

Table 2. Studies with more than 50 patients

Reference	Study type	Patients	Follow Up	PSM
Ballantyne[48]	Registry	498	n.m.	3 (0.6 %)
Bokey[24]	Retrospective	66	median 26 months	1 (1.5 %)
Fielding[28]	Retrospective	149	n.m.	2 (1.3 %)
Fleshman[29]	Registry	372	n.m.	4 (1.1%)
Franklin[22]	Prospective	191	> 30 months	0
Gellman[32]	Retrospective	58	n.m.	1 (1.7 %)
Hoffman[49]	Retrospective	39	> 24 months	0
Huscher[50]	Retrospective	146	mean 15 months	0
Khalili[51]	Retrospective	80	mean 21 months	0
Kwok[35]	Retrospective	83	n.m.	2 (2.5 %)
Leung[36]	Retrospective	179	mean 19 months	1 (0.65%)
Lord[52]	Retrospective	71	mean 16 months	0
Lumley[37]	Retrospective	103	n.m.	1 (1.0 %)
Milsom[53]	Randomized	42	median 18 months	0
Rosato[42]	Registry	1071	n.m.	10 (0.93%)
Vukasin[45]	Registry	480	> 12 months	5 (1.1%)

All studies with more than 50 patients included 3547 patients in total.
In these studies, 30 patients with port-site metastases were found (0.85%).

Table 1. Port-site metastases after laparoscopic resection of colorectal malignancy

Reference	Number of patients and stage of disease		Number and location of psm. (and available details)	Interval (months)
Alexander[12]	1	Duke's C	1: unspec. port	3
Barrat[22]	1	Duke's D	1: unspec. port (diss. dis.)	n.m.
Berends[13]	3	Duke's BII Duke's CII Duke's D	1 psm each patient: 2 umbilical ports, 1 unspec. port	n.m.
Bokey[24]	1	Duke's B	1: RLQ port (excised)	12
Boulez[9]	3	n.m.	n.m.	n.m.
Christen[25]	1	T4NxMx	1: unspec. port	n.m.
Cirocco[26]	1	T3N2Mo	5 : 4 ports + excorp. wound (diss. dis.)	9
Cook[27]	2	Duke's C + liver metas.	1: umbilical port	9
		Duke's C	1: unspec. port	15
Fielding[28]	2	Duke's C	1: unspec. port (diss. dis.)	n.m.
		Duke's D	1: unspec. port (diss. dis.)	
Fingerhut[15]	3	1x Duke's A 2x Duke's B	n.m.	n.m.
Fleshman[29]	4	T4N1Mo	1: conversion laparotomy (diss. dis.)	n.m.
		T3N1Mo	1: unspec. port (excised)	
		T3NoMo	1: unspec. port (excised)	
		T2NoMo	1: unspec. port (excised)	
Fodera[30]	1	n.m.	1: right paraumbilical port	7
Fusco[31]	1	T3N1Mo	1: RLQ port (excised)	10
Gellman[32]	1	Carcinosis	1: unspec. port (diss. dis.)	4
Gionnone (in Wexner[1])	1	Duke's C	1: unspec. port	2
Gould (in Wexner[1])	1	n.m.	n.m.	4
Guillou[14]	1	Duke's C	n.m.	n.m.
Jacquet[33]	2	Duke's C	1: left port	1
		T3NoMo	1: excorporation wound	10
Jacquet[34]	1	T3NoMo	2: right + left port	9
Kwok[35]	2	Duke,s D	1: excorporation wound (diss. dis.)	n.m.
		n.m.	1: unspec. port (diss. dis.)	

Lauroy[16]	1	Duke's A	1: right paraumbilical port	9
Leung[36]	1	n.m.	1: unspec. port (diss. dis.)	n.m.
Lumley [37]	1	Duke's D	1: unspec. port (diss. dis.)	n.m.
Montorsi[38]	1	T3NoMo	1: excorporation wound (excised)	2
Newman (in Wexner[1])	1	Duke's C	1: unspec. port	6
Nduka[39]	1	Duke's C	3: umbilical + right port + perineal wound	3
Ng[40]	1	n.m.	2: umbilical + right port	n.m.
Ngoi (in Wexner[1])	1	Duke's B	1: unspec. port	n.m.
Prasad[17]	2	Duke's B	1: RLQ port (excised)	6
		Duke's A	1: excorporation wound	26
Ramos[41]	3	Duke's C	2: unspec. port + excorp. wound (diss. dis.)	6
		Duke's C	2: unspec. port + excorp. wound (diss. dis.)	8
		Duke's C	1: excorporation wound	21
Rosato[42]	10	2x Stage I 2x Stage II 5x Stage III 1x Stage IV	1 psm each patient : 8x unspec. port (1x diss. dis.) 2x excorporation wound (1x diss. dis.)	Stage I : 18 & 44 rest n.m.
Schaeff[43]	12	8x Stage III 4x Stage IV	1 psm each patient : 8x unspec. port, 3x excorporation wound, 1x perineal wound (7 psm excised)	n.m.
Stitz (in Wexner[1])	1	Duke's D	n.m.	n.m.
Ugarte[44]	1	Duke's C	1: unspec. port	9
Vukasin[45]	5	Stage III	1: excorporation wound	2
		Stage III	1: unspec. port	3
		Stage III	1: unspec. port (2x diss. dis.)	7
		Stage III	1: unspec. port	15
		Stage III	1: excorporation wound	21
Walsh[46]	1	n.m.	1: RLQ port	6
Wexner and Cohen[1]	5	2x Duke's B 3x Duke's C	1 psm each patients: unspec. ports	6-12 3-6
Wilson[47]	1	n.m.	1: unspec. port	n.m.

psm = port-site metastasis, unspec. = unspecified, n.m. = not mentioned,
RLQ = right lower quadrant, excised = psm was excised
diss. dis. = disseminated disease at discovery of psm.

Gallbladder cancer

Laparoscopic cholecystectomy is the most common endoscopic procedure in general surgical practice. The incidence of gallbladder cancer in cholecystectomy specimens varies from 0.18 % to 0.81 % of all cholecystectomies[54]. Although thickening of the gallbladder wall and solitary polipoid lesions greater than 1 cm in diameter can indicate malignancy of the gallbladder, approximately half of all gallbladder cancers is not suspected before surgery. During laparoscopy, small tumors that do not invade the muscular layer, T1 tumors, can easily be missed, particularly because tactile senses are less during laparoscopic surgery. In one study of 24 patients with proven gallbladder cancer, 14 out of 24 patients were not suspected during the operation. Of these 14 cases 9 were classified as either T2 or T3 tumors[55]. When laparoscopic cholecystectomy is performed in those cases with undetected malignancy, tumor cells can be disseminated in the peritoneal cavity due to inadvertent grasping of the tumor or perforation of the gallbladder. The incidence of gallbladder perforation during laparoscopic dissection and removal of the gallbladder has been reported to vary from 24 % to 33 %. In a series of 2616 laparoscopic cholecystectomies, 24 gallbladder cancers were identified, accounting for an incidence of 0.9 %[55]. Three abdominal wall recurrences were observed (13 %), all in patients with either T2 or T3 tumors. In another series of 10925 laparoscopic cholecystectomies, 37 patients had adenocarcinoma of the gallbladder (0.34 %)[56]. Besides one patient, who had a polyp on preoperative ultrasonography of the gallbladder, malignancy was not suspected preoperatively in any of these cases. In 46 % of all gallbladder cancers, malignancy was recognized during operation. Port-site recurrences occurred in 14 % of the patients with gallbladder cancer after a time interval of 6-16 months. These metastases were encountered at equal rates in all tumor stages, but accidental gallbladder perforations significantly increased the chance of port-site recurrences. Suzuki et al. reviewed 3566 patients who had undergone laparoscopic cholecystectomy and 30 patients with unexpected gallbladder carcinoma were identified[57]. Malignancy was noted in only one patient during laparoscopic surgery. Bile spillage occurred in half of the patients with gallbladder cancer. Port-site recurrences were documented in three cases (10 %), staged as T2 and T3 cancers. The 3-year survival rate for T1 disease was 100 %, and for T2 disease 70 %. These rates

appear comparable to those of hidden gallbladder cancer in open surgery[57]. Ricardo et al. reported their experience with 91 patients who were diagnosed with gallbladder cancer[58]. Of these patients, 90 % had advanced disease (T2 or T3 cancers). They analyzed the incidence of port-site recurrences in patients who had laparoscopic cholecystectomy, open cholecystectomy, or converted procedures. In advanced gallbladder cancer, the operative technique appeared not to affect the rate of abdominal wall recurrence. One patient in this study presented with an umbilical metastasis prior to surgery. Such rare umbilical metastases from intra-abdominal tumors are known as 'Sister Mary Joseph's nodules' and can be the first sign of malignant disease in a patient[59]. Though its pathogenesis may be different from the pathogenesis of port-site recurrences, the 'Sister Mary Joseph's nodule' in this patient illustrated the avidity of gallbladder cancer cells for the abdominal wall. In this laparoscopic era, thorough ultrasonographic evaluation of the gallbladder is mandatory to prevent any dismal consequences of laparoscopic cholecystectomy. In suspect cases, laparoscopy can be performed to assess the macroscopic appearance of the gallbladder. When the serosal aspect of the gallbladder appears normal, laparoscopic ultrasonography should be performed subsequently to identify abnormal thickening of the gallbladder wall, polyps or enlarged lymph nodes in the hepatoduodenal ligament. In case of any remaining suspicion, laparoscopy should be converted to open surgery.

Urological Malignancy

Laparoscopy plays an important role in the diagnosis and treatment of urological malignancy. In several centers with laparoscopic and oncological expertise, laparoscopic nephrectomy has become the procedure of choice in patients with renal cell cancer staged as T1 or T2. In a registry of 157 patients who had undergone laparoscopic radical nephrectomy, abdominal wall recurrence was not observed at a mean follow-up period of more than 2.5 years[60]. Obviously, a longer follow-up is mandatory to draw final conclusions about the incidence of abdominal wall recurrence, since renal cell cancer is known for its late metastases. Of interest is the technique for specimen removal in laparoscopic radical nephrectomy. Unlike general surgeons, urologists do not refrain from morcellating cancerous specimens in a plastic bag. In spite of this technique, port-site recurrences have not been reported after laparoscopic radical nephrectomy. In the registry of 157 patients, only

one patient had a local recurrence located in the ureteral stump. Retrograde embolization of tumor cells or implantation of exfoliated renal cells were considered possible mechanisms of the ureteral recurrence. Similar ureteral recurrence after radical nephrectomy has been reported after open procedures[61]. Therefore, laparoscopy does not appear instrumental in ureteral recurrence. Laparoscopic pelvic lymphadenectomy is commonly employed to assess the lymphatic spread of prostatic cancer. In this procedure lymph nodes are frequently removed without use of a protecting bag. Surprisingly, only few reports exist on port-site recurrences after laparoscopic pelvic lymphadenectomy[62, 63]. Direct laparoscopic biopsy of bladder cancer was followed by a port-site recurrence[64]. Janetschek et al. reported that they did not observe any port-site recurrences in 24 patients who had had laparoscopic lymphadenectomy for non-seminomatous testicular cancer at a mean follow-up of two years[65]. Non-seminomatous testicular cancer requires chemotherapy prior to abdominal lymphadenectomy. Thus, possibly, chemotherapy reduced the risk of port-site recurrences.

Diagnostic Laparoscopy

Diagnostic laparoscopy, frequently in combination with laparoscopic ultrasonography and biopsy, is a valuable tool to identify the cause of ascites or assess the resectability of abdominal cancers. It may prevent unnecessary laparotomies in up to 32 % of patients with gastrointestinal malignancies, or modify the treatment in up to 34 %[23,66,67]. However, the occurrence of port-site recurrences after diagnostic laparoscopy raises the question if this procedure in known or suspected malignancy, exposes patients with resectable malignant disease to the risk of cancer dissemination. Reports on port-site recurrences after diagnostic laparoscopy are scarce and the population of patients is seldom mentioned. Furthermore, there is considerable heterogenity of underlying diseases in the studied patients. Nieveen-Van Dijkum et al. described in 1996 a series of 250 patients who had undergone a laparoscopy for staging of gastrointestinal malignancy, including 121 periampullary tumors, 66 esophageal tumors, 26 proximal bile duct cancers, 21 liver tumors, and 13 other intra-abdominal malignancies[68]. Four of these patients (1.6 %) developed a port-site recurrence. In two of these patients, intraoperative biopsy showed metastatic disease, but in the other two, no metastases were seen. These latter two patients under-

went exploratory laparotomy which revealed locally advanced non-resectable tumors. Interestingly, metastases developed at the port-sites, but not in the laparotomy wound. In another study, 3 out of 40 patients undergoing laparoscopy for staging (40 esophageal cancers and 10 gastric cancers) developed port-site recurrences (7.5 %)[27]. Two patients had gastric carcinoma (20 % of all gastric cancers) and one patient had esophageal cancer (3.3% of all esophageal cancers). In a French study of 109 patients with gastrointestinal malignancy, no port-site recurrences after diagnostic laparoscopy with intra-abdominal ultrasonography were reported[23]. A British series reported 49 patients who underwent diagnostic laparoscopy for gastric cancer[66]. Port-site recurrence occurred in one patient with intraoperatively assessed disseminated disease (2 %). In a large Dutch study of 286 patients that had laparoscopy and laparoscopic ultrasonography for periampullary and pancreatic cancers, 7 patients with port-site recurrences were identified (2.4 %)[67]. All patients had disseminated disease at the time of laparoscopy and port-site tumors developed after a mean period of 8 months. Although all port-site recurrences after a diagnostic procedure were seen in patients with non-resectable tumors or disseminated disease, some of them required excision for patient comfort. Muensterer et al. Recommended midline placement of trocars, which should facilitate port-site excision at the time of cytoreductive surgery[69].

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CHAPTER 3

PORT-SITE METASTASES AFTER CO_2 LAPAROSCOPY IS AEROSOLIZATION OF TUMOR CELLS A PIVOTAL FACTOR?

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ABSTRACT

Background

Animal experiments showed that CO_2 laparoscopy results in more port-site recurrences than gasless laparoscopy. Possible transport by CO_2 of aerosolized tumor cells was investigated in rats.

Methods

Abdominal cavities of 15 pairs of WAG rats were connected and $2x10^6$ or $16x10^6$ CC-531 cells were injected in the first (donor) rat of each pair. Ten liters of CO_2 were allowed to flow from the first (donor) to the second (recipient) rat.

Results

No tumor was found in the recipients after injection of $2x10^6$ cells in the donors. Injection of $16x10^6$ cells in the donors resulted in very limited tumor growth in the recipients.

Conclusions

Aerosolization of tumor cells occurs, but the required number of intraperitoneal tumor cells to result in metastases by this mechanism is extremely high. Therefore, aerosolization of tumor cells appears not of major relevance in the pathogenesis of port-site metastases.

INTRODUCTION

Laparoscopic surgery for colon carcinoma has caused controversy among colorectal and laparoscopic surgeons since its introduction in 1991. The main adverse consequence of this new surgical approach to colon cancer was the occurrence of metastases at port-sites[1,2]. Recent studies on laparoscopic resection of colorectal cancer have reported incidences of port-site metstases which seem comparable to the incidence of wound metastases in conventional surgery[3-5], but port-site metastases remain a clinical important concern. Although various pathways for development of port-site metastases have been suggested, prevention of these recurrences is still thwarted by shortcoming understanding of their pathogenesis. Direct implantation of tumor cells in port-site wounds appears more plausible than transport of tumor cells via blood or lymphatic vessels to the port-sites[1]. One of the causes of direct implantation could be transportation of tumor cells by the insufflated gas to the port-sites. This possibility has drawn great interest since experimental work showed that gasless laparoscopy resulted in fewer port-site metastases than conventional laparoscopy, suggesting a special role of the pneumoperitoneum in the pathogenesis of these recurrences[6-8]. During CO_2 laparoscopy, whirling and light reflecting particles are commonly visible. Macroscopically, these particles appear to consist of droplets and fibrous structures. It seems likely that malignant cells, either solitary, as clumps or attached to particles, can be displaced in the peritoneal cavity in the same fashion[9]. This hypothetical situation of tumor cells floating in the insufflated abdominal cavity is designated as aerosolization of tumor cells[10]. Floating of cells in the peritoneal cavity would be caused and maintained by turbulence due to insufflation and leakage of gas, tissue manipulation, coagulation[9] and changing of instruments in the ports. Although this mechanism of dispersal of tumor cells in laparoscopy appears obvious, aerosolized tumor cells can be difficult to detect[9,11-13]. In an attempt to evaluate the role of aerosolization of tumor cells in development of port-site metastases, we used a model in which an insufflated tumor bearing abdominal cavity of a rat was connected to a non tumor bearing abdominal cavity of a host.

MATERIALS AND METHODS

Animals

Thirty male rats of the inbred WAG/Rij (Wistar Agouti / Rijswijk) strain, weighing 250-300 g and bred under specific pathogen-free conditions (Harlan, Austerlitz, The Netherlands), were used. At our labaratory, they were kept under the following conditions: temperature of 20-24 °C, relative air humidity of 50-60 % and a 12 hours light-12 hours dark cycle. The animals had free access to water and food (Hope Farms, Woerden, The Netherlands) before and after surgery. The protocol was approved of by the Committee on Animal Research of Erasmus University, Rotterdam, The Netherlands.

Tumor

CC-531 is a moderately differentiated colonic adenocarcinoma, chemically (1,2-dimethylhydrazine) induced in the WAG strain. This tumor is tranplantable in syngeneic WAG rats and weakly immunogenic as determined by the immunization challenge method described by Prehn and Main[14]. The tumor was kept *in vitro* in RPMI 1640 medium with 5 % fetal calf serum (virus and mycoplasm screened), 1 % penicillin (5000 U/mL), 1% streptomycin (5000 U/mL) and 1 % L-glutamine (200 mmol/L). All supplements were obtained from Gibco, Paisley, UK. For preparation of the tumor cell suspension, cells were trypsinized (5 min. at 37 °C), centrifuged (5 min 3000 rpm), resuspended in RPMI 1640 and counted. Viability of more than 95 % of cells was checked by trypan blue staining (0.3 % in 0.9 % sodium cloride solution). A suspension of $1x10^6$ cells per mL (group 1) and of $8x10^6$ cells per mL (group 2) was prepared and stored on ice untill injection in the rats.

Instruments

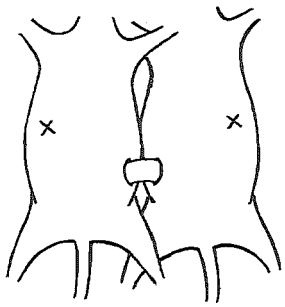
A closed box which could be connected to ether-saturated air was used for induction of anaesthesia in the rats. Anaesthesia was maintained with ether which evaporated from a plug of cotton in a tube placed over the snout of the rats. An automatic insufflator (Van Straaten Medical, Nieuwegein, The Netherlands) established a pneumoperitoneum with room temperature CO_2. Disposable 5 mm trocars (Surgiport, US Surgical, Norwalk, CT,

USA) were shortened for use in rats. A transparant poly vinyl chloride tube for connecting abdominal cavities of rats had a length of 2.5 cm and a lumen diameter of 7 mm.

Procedure

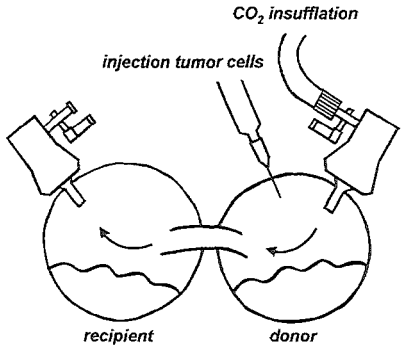
After induction of anaesthesia in 2 rats, their abdomens were shaved. They were positioned supine, close to one another and secured with tape to the operative table. One 5 mm trocar was introduced through a 5 mm incision in the left upper quadrant of one rat and another 5 mm trocar was introduced in the right upper quadrant of the other rat (fig. 1). Both trocars were secured with purse string sutures. In the right lower quadrant of the right rat and in the left lower quadrant of the left rat, an incision of 1 cm was made. Each end of the connecting tube was introduced in these incisions and secured gastight with purse string sutures. In these connected abdominal cavities, a pneumoperitoneum with a pressure of 6 mm Hg was established via the trocar of the rat on the right side (fig. 2).

Figure 1.



Position of the rats with the connecting tube in situ. The crosses mark introduction sites of the 5 mm trocars.

Figure 2.



Schematic cross-sectional view of the experimental setup in two rats. The tube is connecting the abdominal cavities of donor and recipient rat.

To prevent transportation of liquid between the abdominal cavities of the rats during the entire experiment, the introduction sites of the connecting tube were at least 1½ cm higher than the lowest point in the peritoneal cavity of the rats in supine position. In order to avoid drainage of a fluid film lining the peritoneal surface of the abdominal wall into the connecting tube (like in a sink), the tube ends extended one cm into the peritoneal cavity of each rat (fig. 2). Laparoscopic inspection of both peritoneal cavities was done to assure that the ends of the connecting tube were not in direct contact with the abdominal contents. The rat on the right side is referred to as the 'donor' and the rat on the left side as the 'recipient'. The CC-531 tumor cell suspension ($2x10^6$ in 2 mL medium in group 1 and $16x10^6$ in 2 mL medium in group 2) was injected in the abdominal cavity of the donor. Subsequently, a gas flow was induced through the system by partly opening the stopcock of the trocar in the recipient. Ten liters of CO_2 were allowed to leak in 10-12 minutes. At the end of the procedure, care was taken to avoid spill of intraperitoneal fluid from the donor to the recipient through the connecting tube in the following way. The pneumoperitoneum was relieved via the trocar in the donor and subsequently the connecting tube was extracted from the recipient. Port-sites were closed with 3-0 silk sutures. Group 1 consisted of 20 rats and group 2 consisted of 10 rats. Evaluation of tumor growth was performed via median laparotomy.

RESULTS

Group 1

Three weeks postoperatively, all 20 rats were analyzed for tumor growth. In all donor rats 1-2½ cm tumor deposits at the port-sites and abundant intraperitoneal tumor growth were found. All 10 donors were terminated. In 4 of 10 recipients, 6 small (1-2 mm) suspect structures were identified. Locations of these structures were noted and the laparotomies were closed. At a second look procedure 3 weeks later, these structures had not grown which indicated a benign character. No other suspect lesions were found at this second look procedure (Table).

Group 2

Two weeks postoperatively, all donor rats were terminated. As in the first experiment, there were 1-2½ cm tumors at the port-sites and multiple tumors throughout the entire peritoneal cavity. Recipients were checked 4 weeks postoperatively. In 3 of 5 rats there were solitary tumors with a diameter of 2-4 mm at the 'port-site' of the connecting tube. Histopathologic examination of these tumors revealed adenocarcinoma tissue (Table).

Table of results

	Group 1 ($2x10^6$ CC-531 cells)		Group 2 ($16x10^6$ CC-531 cells)	
Rats:	Donors: 10	Recipients: 10	Donors: 5	Recipients: 5
Tumor growth:	Abundant & ascites in all	None	Abundant & ascites in all	Small solitary tumors in 3

DISCUSSION

The primary question addressed in this study was whether tumor cells could be transported by gas in an aerosolized state and at the same time retain their ability to proliferate after deposition elsewhere. In order to demonstrate aerosolized tumor cells, different experimental setups have been applied. Thomas et al.[12] used a container with a tumor cell suspension and a filter attached to one of the ports. No tumor cells were identified in the filters, whereas instruments and ports were greatly contaminated with malignant cells. In an experiment with pigs, Hewett et al.[11] found similar results. Malignant cells were only present on instruments, on ports and on one filter attached to a port used for instrumentation. Champault et al.[9] performed a study in 9 humans that underwent a variety of laparoscopic procedures for benign and malignant disorders. Benign cells were recovered from 6 of 9 filters, which at least suggests that cells, benign or malignant, can be carried to the ports by gas insufflation. On the other hand, Whelan et al.[13] used culture media in stead of filters to detect malignant cells in an *in vitro* and an *in vivo* study and observed no tumor growth in culture dishes. The drawback of the aforementioned studies is that "unnatural" media were used for tumor cell collection and culture. The most natural medium, which also reflects the clinical situation, is the abdominal cavity. Therefore we used an acceptor rat as medium in this study.

In the first experiment we injected 4 times the usual tumor load ($5x10^5$ cells) that we used in WAG rats in preliminary studies[6,15]. Assuming that this tumor load was still insufficient, we repeated the experiment with 32 times the usual tumor load ($16x10^6$ CC-531 cells) in the donor rats of group 2. In spite of this excessive amount, it resulted in very limited tumor growth in the recipient. As a theoretical illustration to support the statement that $16x10^6$ CC-531 cells is an extreme tumor load, we calculated that $16x10^6$ tumor cells in a rat equals a volume of about 0.83 cm^3 of tumor cells dispersed in a human abdominal cavity (see Specification). In case of uncomplicated resection of tumors without invasion of the serosa, such a spill seems unrealistic. However, port-site metastases do develop in these cases, which suggests that other mechanisms than aerosolization are responsible. This study shows that aerosolization of viable tumor cells is possible. However, an extremely high number of free intraperitoneal cells is needed to result in metastases.

In other experimental designs, port-site metastases occurred in the presence of much smaller numbers of free tumor cells, probably by other routes. Therefore, we conclude that aerosolization is unlikely to be an important factor in the pathogenesis of port-site metastases.

The fact that CO_2 laparoscopy is associated with a higher incidence of port-site metastases than gasless laparoscopy in experimental studies, is to be found in other gas related factors. The 'chimney effect' has been suggested as a possible mechanism of spreading tumor cells to port-sites[8]. The chimney effect refers to the increase of the number of tumor cells at the port-sites due to leakage of gas along trocars. Tseng et al.[16] reported increased tumor growth at leaking port-sites in a rat study. Considering the result of the present study, it appears likely that tumor cells transported in droplets or in the fluid film that is lining the peritoneal cavity were the cause of increased tumor growth at the leaking port-site. In addition to constant leakage of gas, concern exists about inadvertent trocar removal or deflating the pneumoperitoneum by extracting a trocar. In both instances, a sudden high flow of gas escapes the abdomen through the port-site and frequently a spray of droplets can be noted. Essentially, this condition represents the chimney effect in an extreme form and it appears likely that it predisposes to tumor growth at the port-site.

In conclusion, aerosolized tumor cells appear to play a minor role in the development of port-site metastases. However, tumor cells in droplets are a potential hazard. Therefore, leakage of gas along trocars and sudden removal of trocars should be avoided.

Specification

At an insufflation pressure of 6 mm Hg, the mean intraperitoneal volume of the rats was 50 mL. The intraperitoneal volume in humans, when a pressure of 12 mm Hg during laparoscopy is applied, is about 3 L. So the human intraperitoneal volume is about 60 times that of a rat . In group 2 of this experiment, $16x10^6$ cells were injected intra-peritoneally. To equal this concentration of free intraperitoneal tumor cells, $9.6 x10^8$ cells ($60 x 16x10^6$) are needed in a human abdominal cavity. $9.6x10^8$ cells weigh about 960 mg and have a volume of about 0.83 cm^3 solid tumor tissue.

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CHAPTER 4

INTRAPERITONEAL TUMOR GROWTH IS INFLUENCED BY PRESSURE OF CO_2 PNEUMOPERITONEUM.

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ABSTRACT

Background

Several studies indicated that the CO_2 pneumoperitoneum during laparoscopy plays a role in the pathogenesis of port-site metastases. An experimental animal study was performed to investigate the impact of various pressures of the pneumoperitoneum on peritoneal tumor growth.

Methods

36 male WAG rats were randomized to 3 groups with different pneumoperitoneum pressures: 16 mm Hg, 4 mm Hg and gasless controls. After establishing the pneumoperitoneum, $0.5x10^6$ CC-531 tumor cells were injected intra-abdominally and the pneumoperitoneum maintained for 60 minutes. Peritoneal tumor growth was assessed on day 11 at autopsy.

Results

Peritoneal tumor growth in the the 16 mm Hg group was significantly greater than in the 4 mm Hg group ($p = 0.039$) and the gasless group ($p = 0.004$).

Conclusions

High pressure CO_2 pneumoperitoneum stimulates intra-abdominal tumor growth. Employing low insufflation pressures in laparoscopic cancer surgery should be considered.

INTRODUCTION

Laparoscopic surgery in oncologic patients is increasingly adopted as an alternative to conventional surgical procedures, both for diagnosis and resection. Studies on laparoscopic resections in patients with colorectal carcinoma showed no difference in resection margins or lymph node harvest when compared to conventional surgery[1]. Moreover, many of these studies suggest that short term benefits which are associated with laparoscopic cholecystectomy, such as less pain, less wound complications and a shorter recovery, are also seen after colorectal laparoscopic procedures[2]. The main concern which gloomed at the start of laparoscopic cancer surgery, was the occurrence of port-site metastases[3]. Judging from published registries and retrospective studies with large numbers of patients, the incidence of these recurrences seems far less than initial reports suggested[4]. However, port-site metastases are still a clinical important problem. Particularly the fact that in some instances the pathogenesis of port-site metastases remains uncomprehensible, raises questions on the influence of the CO_2 pneumoperitoneum on tumor cell and host biology. An intriguing observation in animal experiments is the finding that CO_2 laparoscopy was associated with more peritoneal tumor growth than gasless laparoscopy[5,6]. We wondered if the stimulatory impact of CO_2 insufflation on tumor growth would be proportional to the insufflation pressure. To investigate this possibility, we designed an animal experimental study to evaluate the impact of CO_2 pneumoperitoneum at different pressures on intraperitoneal tumor take.

MATERIALS AND METHODS

Animals

Thirty-six male rats of the inbred WAG/Rij strain (Wistar Agouti / Rijswijk), weighing 250-300 g and bred under specific pathogen-free conditions (Harlan, Austerlitz, The Netherlands), were kept under the following conditions: temperature of 20-24 °C, relative air humidity of 50-60 % and a 12 hours light-12 hours dark cycle. The animals had free access to water and food (Hope Farms, Woerden, The Netherlands) before and after sur-

gery. The protocol was approved of by the Committee on Animal Research of Erasmus University, Rotterdam, The Netherlands.

Tumor

CC-531 is a moderately differentiated colonic adenocarcinoma, chemically (1,2-dimethylhydrazine) induced in the WAG strain. This tumor is transplantable in syngeneic WAG rats and weakly immunogenic as determined by the immunization challenge method described by Prehn and Main. The tumor was kept *in vitro* in RPMI 1640 medium with 5 % fetal calf serum (virus and mycoplasm screened), 1 % penicillin (5000 U/mL), 1% streptomycin (5000 U/mL) and 1 % L-glutamine (200 mmol/L). All supplements were obtained from Gibco, Paisley, UK. For preparation of the tumor cell suspension, cells were trypsinized (5 min. at 37 °C), centrifuged (5 min 3000 rpm), resuspended in RPMI 1640 and counted. Viability of more than 95 % of cells was checked by trypan blue staining (0.3 % in 0.9 % sodium cloride solution). A suspension of $0.5x10^6$ cells per mL was prepared and stored on ice untill injection in the rats.

Instruments

A closed box which could be connected to ether-saturated air was used for induction of anesthesia in rats. Ether anesthesia was maintained during the experiment with an ether-saturated plug of cotton in a tube placed over the snout of the rats. An automatic insufflator (Van Straaten Medical, Nieuwegein, The Netherlands) established a pneumoperitoneum with room temperature CO_2. Disposable 5 mm trocars (Surgiport, US Surgical, Norwalk, CT, USA) were shortened for use in rats.

Procedure

Thirty-six rats were numbered and randomized to 3 different groups: 4 mm Hg CO_2 pneumopertioneum, 16 mm Hg CO_2 pneumoperitoneum or 0 mm Hg pneumoperitoneum (gasless control). Rats were anaesthetized, abdomens were shaved and they were secured with adhesive tape in a supine position to the operating table. Two 5 mm trocars were introduced with an open technique, left and right in the abdomen and secured with purse string sutures. In the 4 and 16 mm Hg pressure groups, the rats were attached to the insuf-

flator at one of the trocars and the pneumoperitoneum was established. In rats of the 0 mm Hg pressure group, both trocars were suspended with light traction to a laboratory stand, thus creating an intra-abdominal working space. After establishing the pneumoperitoneum, $0.5x10^6$ CC-531 cells in one mL were injected intraperitoneally through the midline. The pneumoperitoneum lasted for 60 minutes and after this period, trocars were removed and wounds were closed. In order to preclude a time bias during this 'operative' phase of the experiment, the three groups were treated synchronically. After their treatment, rats were placed randomly in cages of three animals. From this moment, the investigators were blinded to the treatment of each rat. To prevent overgrowth of tumor tissue, which would compromise the assessment of tumor take, tumor growth was checked every day by palpating the tumor mass at the port-sites. All rats were sacrificed on day 11. Abdomens were opened by a T-shaped laparotomy, tumor masses at port-sites were dissected and weighed. Tumor take at other intraperitoneal sites was assessed semi quantitatively at six locations (see specification). Only after assessment of tumor growth in all the animals, the numbers of the rats were linked to the three different treatments (0,4 and 16 mm Hg during 60 min.) Tumor mass and tumor score of the 3 groups were compared and analyzed by the Mann-Whitney test.

RESULTS

One rat in the gasless group died during the experiment, probably as a result of respiratory depression caused by excessive ether inhalation.

The mean tumor mass at both port-sites per rat was 1133 mg in the gasless group, 1120 mg in the 4 mm Hg pressure group and 1213 mg in the 16 mm Hg pressure group. Differences between the groups were not significant.

The mean total intraperitoneal tumor growth (tumor growth at port-sites excluded) was 9.45 in the gasless group, 10.83 in the 4 mm Hg pressure group and 13.33 in the 16 mm Hg pressure group (Table 1). The differences between the gasless and the 16 mm Hg pressure group ($p = 0.004$) and between the 4 mm Hg pressure group and the 16 mm Hg pressure group ($p = 0.039$) were significant (Table 2). In all groups, tumor take at the parietal peritoneum was scant.

Table 1. Mean tumor take at different sites and mean total tumor load

	0 mm Hg (n = 11)	4 mm Hg (n = 12)	16 mm Hg (n = 12)
Kidney R	0.55 ± 0.28	1.50 ± 0.26	1.92 ± 0.29
Kidney L	0.55 ± 0.21	1.00 ± 0.28	1.58 ± 0.29
Mesentery	3.00 ± 0.00	2.83 ± 0.11	2.83 ± 0.11
Liver	1.82 ± 0.33	1.58 ± 0.29	2.42 ± 0.23
Retroperitoneum	1.55 ± 0.21	2.08 ± 0.23	2.58 ± 0.19
Scrotal fat	2.00 ± 0.23	1.83 ± 0.21	2.00 ± 0.12
Total tumor load	9.45 ± 0.93	10.83 ± 0.77	13.33 ± 0.68

Figures in the table are the mean tumor score ± S.D, according to a semi-quantitative scoring system.

Table 2. P- values of differences

	0 *vs.* 4 mm Hg	0 *vs.* 16 mmHg	4 *vs.* 16 mm Hg
Kidney R	0.023	0.007	>0.2
Kidney L	>0.2	0.016	0.18
Mesentery	>0.2	>0.2	>0.2
Liver	>0.2	>0.2	0.05
Retroperitoneum	0.13	0.004	0.14
Scrotal fat	>0.2	>0.2	>0.2
Total tumor load	>0.2	0.004	0.039

P-values of differences in peritoneal tumor take at different pressures.

DISCUSSION

Port-site metastases have been subject of many experimental studies. In spite of these research efforts, some causes of port-site recurrences remain unclear. One of the most likely mechanisms that emerged from several experiments, is implantation of malignant cells at port-sites by laparoscopic instruments bearing viable tumor cells[7,8]. Another evident mechanism is tumor cell implantation in the abdominal wall when a tumor is extracted through a narrow unprotected wound[9]. However, these modalities do not account for all port-site recurrences. All other suggested mechanisms of port-site metastases are related to the CO_2 pneumoperitoneum.

In this study, we focussed on the role of insufflation pressure on peritoneal tumor growth. The important finding was that a pneumoperitoneum of 16 mm Hg was associated with greater tumor growth than a pneumoperitoneum of 4 mm Hg. Various factors of high pressure CO_2 laparoscopy may contribute to the stimulating effect on tumor take. Schilling et al.[10] reported a significant decrease in microcirculatory splanchnic blood flow when the pressure of the pneumoperitoneum increased from 10 to 15 mm Hg in a clinical study. There was no significant difference in blood flow between 0 and 10 mm Hg pressure. Because the blood pressure in rats, as in most mammals used for experiments, is comparable to the blood pressure in humans, we felt that the use of rats would not compromise the validity of our results when extrapolating these to the human situation. In our animal experiment, the increased intraperitoneal tumor take possibly reflects decreased microcirculation with resulting ischemia. Ischemia could render the peritoneum more susceptible to tumor growth. High pressure pneumoperitoneum might also exert its influence through a systemic effect. Mikami et al.[11] compared the influence of a pneumoperitoneum with air, N_2O and CO_2 at both 10 mm Hg and at 20 mm Hg on catecholamine plasma concentrations in pigs. An increase in pressure to 20 mm Hg resulted in a significant increase in plasma catecholamine concentration with all gases, whereas a pressure of 10 mm Hg had no effect.

The finding that a 16 mm Hg CO_2 pneumoperitoneum results in greater tumor growth than a 4 mm Hg pneumoperitoneum can have important clinical consequences. Although complete avoidance of peritoneal gas insufflation appears preferable, performing gasless

laparoscopic colectomy is very difficult, particularly in obese patients. However, laparoscopic surgery at lower pressures (4-8 mmHg) can provide adequate visibility and seems more feasible than gasless surgery. Therefore, low pressure laparoscopy in colorectal cancer surgery will be more acceptable to adopt for most laparoscopic surgeons than gasless surgery. It is still unclear if a direct relation between intraperitoneal pressure and tumor growth exists or if there is a 'threshold' pressure. Further studies are necessary to determine the optimal insufflation pressure.

Specification semi quantitative scoring system

Tumor take was assessed at six intra-abdominal locations: the right kidney, the left kidney, the mesentery, the liver, the retroperitoneal surface and scrotal fat. Two independent observers, blinded for the treatment of the animals, estimated tumor take at each location according to the following scoring system: 0: no tumor take, 1: minor tumor take, 2: moderate tumor take and 3: extensive tumor take. In case of disagreement between the observers, the score was middled. No definition of minor, moderate or extensive tumor take could be given because the difference in peritoneal surface of the locations and uncomparability of pattern and amount of tumor take at each location. In general, a score of 1 represented a few pin point lesions and a score of 3 represented many lesions with a diameter of > 2 mm or a tumor mass with a diameter of > 5 mm.

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CHAPTER 5

INCREASED TUMOR GROWTH AFTER HIGH PRESSURE PNEUMOPERITONEUM WITH HELIUM AND AIR.

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ABSTRACT

Introduction

Tumor growth appears proportional to the pressure of carbon dioxide insufflation during laparoscopic surgery. Air and helium are alternative insufflation gases. The objective of this study is to assess tumor growth after air and helium insufflation at different pressures.

Method

Ninety-six WAG rats were allocated to either air or helium. In both arms, rats were randomly exposed to a one hour gasless procedure, 4 mm Hg, 10 mm Hg or 16 mm Hg insufflation. At the start of the procedure, 500,000 CC-531 tumor cells were injected intraperitoneally. After three weeks, intraperitoneal tumor growth was assessed.

Results

Higher insufflation pressures were associated with greater tumor growth. No difference of tumor growth between air and helium insufflation was found.

Discussion

In this experimental model, insufflation pressure appeared to have a greater impact on tumor growth than the type of gas. Further studies are necessary but it seems prudent to recommend employment of lower insufflation pressures in laparoscopic oncologic surgery.

INTRODUCTION

Port-site metastases can occur after laparoscopic surgery for malignancy. Although abdominal wall recurrence after conventional cancer surgery has been reported for more than one century, the occurrence of port-site metastases demands thorough studies on their pathogenesis. A major difference between open and laparoscopic surgery is insufflation of the abdomen. As a consequence, the impact of various types of gas, insufflation pressure, temperature and humidity of the insufflation gas have been studied. In a previous experimental study[1], it was shown that tumor growth was increased at higher pressures of carbon dioxide pneumoperitoneum. Carbon dioxide is readily absorbed by peritoneum and blood causing local and systemic acidosis. Intraperitoneal carbon dioxide insufflation affects the integrity of the peritoneum and interferes with metabolic and immune responses[2-5].

Alternative gases for insufflation are helium and air. Helium is an inert gas while air is a mixture of nitrogen and oxygen. The objective of this experimental study was to assess tumor growth after insufflation of air and helium at different pressures.

MATERIALS AND METHODS

Ninety-six WAG/Rij rats, weighing 200-220 g, were allocated to two groups: 48 rats in the air study and 48 rats in the helium study. In both studies, groups of 12 rats were exposed to 4 different procedures: gasless laparoscopy (which is essentially a 0 mmHg pneumoperitoneum) serving as control, 4 mm Hg, 10 mm Hg and 16 mm Hg gas insufflation. To reduce discrepancy between tumor cells, tumor cell batches were equally distributed among gasless, 4 mm Hg, 10 mm Hg and 16 mm Hg groups. Rats were anesthetized by ether inhalation and randomly numbered. In rats undergoing gasless laparoscopy, the abdominal wall was elevated by light traction of elastic bands attached to two subcutaneous needles in the left and right abdomen. All rats had two 5 mm trocars which were introduced and secured with a purse string in the left and right half of then abdomen. The duration of the pneumoperitoneum was one hour in all groups. After establishing a pneu-

moperitoneum by abdominal wall elevation or gas insufflation, 500,000 CC-531 cells suspended in 1 mL of RPMI 1640 culture medium were injected intraperitoneally through the abdominal midline in all groups. After 1 hour, the trocars were removed and the wounds were closed in one layer by a single suture.

Three weeks after the procedure, all animals were sacrificed to evaluate intra-abdominal tumor growth according to the following semi-quantitative scoring system. Tumor take was assessed at nine intra-abdominal locations: right kidney, left kidney, retroperitoneal surface, hepatic hilum, hepatic surface, diaphragm, mesentery, omentum, and scrotal fat. Two independent observers assessed tumor take at each location according to the following scoring system: 0: no tumor take, 1: minor tumor take (pin point tumor deposits), 2: moderate tumor take and 3: extensive tumor take (tumor deposits with a diameter >2 mm or a tumor mass with a diameter > 5 mm). In case of disagreement between the observers, the mean of the individual scores was used. The scores of all 9 locations composed the total tumor score. The tumor masses at the trocar wounds were dissected and weighed.

Results were first statistically analyzed for each group independently. Secondly, data of the 4 mm Hg and 10 mm Hg groups were combined in each study, since the tumor scores for these groups were very similar. Finally, data of both studies were combined to discern differences in tumor scores between gases and pressure groups. Hereto, an ANOVA model was constructed incorporating gas (air/helium), pressure (gasless, 4+10 mm Hg, 16 mm Hg), and day of treatment as covariates. Comparisons between pressure groups (gasless vs. 4+10 mm Hg, 4+10 mm Hg vs. 16 mm Hg) were performed with Dunnett's T-tests, which correct for multiple comparisons.

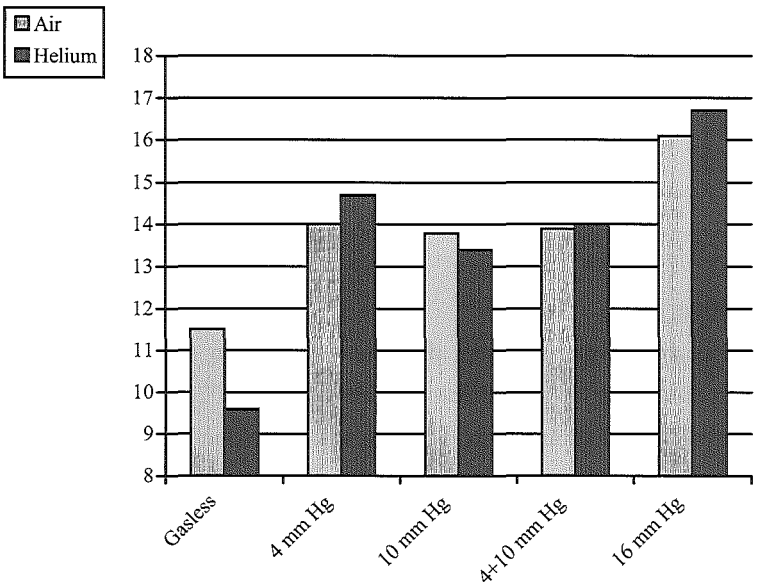
RESULTS

Two animals died during the procedure: one in the gasless (0 mm Hg) group of the air study and another in the 16 mm Hg group of the helium study. These two animals were not included in the analysis. Total tumor scores for all groups are shown in table 1 and in the figure.

Table 1. Total tumor scores

Group	Air	n	Helium	n
Gasless	11.5 (± 4.9)	11	9.6 (± 4.6)	12
4 mm Hg	14.0 (± 4.0)	12	14.7 (± 5.0)	12
10 mm Hg	13.8 (± 4.6)	12	13.4 (± 3.8)	12
4+10 mm Hg	13.9 (± 4.2)	(24)	14.0 (± 4.4)	(24)
16 mm Hg	16.1 (± 4.6)	12	16.7 (± 4.0)	11

Figure. Total tumor scores for air and helium



Air study

Univariate ANOVA corrected for treatment day showed no significant differences of total tumor score related to pressure (Table 2). However, when data of the 4 and 10 mm Hg pressure group were combined, the same analysis showed that tumor growth was significantly greater at higher insufflation pressures ($p = 0.05$, Table 2).

Helium study

Univariate ANOVA corrected for treatment day showed significantly greater tumor growth at higher insufflation pressures ($p = 0.003$, Table 2). Combining data of the 4 and 10 mm Hg pressure group confirmed that tumor growth was proportional to insufflation pressure ($p = 0.001$, Table 2).

Table 2. ANOVA analysis of tumor scores related to pressure and gas.

Analyzed factors	Air (n=47)	Helium (n=47)	Air + Helium (n=94)
0 - 4 - 10 - 16 mm Hg	n.s.	0.003	0.026
0 - (4+10) - 16 mm Hg	0.05	0.001	0.012
Air - Helium	-	-	n.s.

Combined air and helium study

Univariate ANOVA corrected for treatment day and focusing on possible differences related to type of gas, demonstrated a significant difference ($p = 0.026$) between insufflation at different pressures when air and helium groups were combined, but no differences of tumor growth in rats which had either air or helium insufflation (Table 2).

A Dunnett's test analysis of pressure related differences of tumor growth in the combined air and helium groups was performed. Differences between the gasless (0 mm Hg) and 4 mm Hg group were significant ($p = 0.008$), as well as differences between the 0 mm Hg

and 10 mm Hg group ($p = 0.043$) and between the 0 mm Hg and 16 mm Hg group ($p < 0.000$, Table 3). The combined 4 mm Hg and 10 mm Hg groups had significantly less tumor growth than the 16 mm Hg group ($p = 0.049$, Table 3).

Table 3. Dunnett's analysis of tumor scores related to pressure of the air and helium groups combined.

Compared groups			p
Gasless	*vs*	4 mm Hg	0.008
	vs	10 mm Hg	0.043
	vs	16 mm Hg	< 0.000
16 mm Hg	*vs*	4 mm Hg	n.s.
	vs	10 mm Hg	n.s.
	vs	4+10 mm Hg	0.049

DISCUSSION

Carbon dioxide insufflation has been suggested as an important cause of port-site metastases. Insufflating gas into a peritoneal cavity which contains free viable tumor cells could displace tumor cells to the port-sites predisposing to port-site metastases. Leakage of gas along trocar shafts, known as the 'chimney effect'[6], could increase the number of tumor cells at the port-sites and subsequently predispose to port-site metastases. Besides these effects related to gas turbulence in the abdomen, intrinsic effects of CO_2 are known. Jacobi et al. showed that carbon dioxide incubation of tumor cells stimulates growth[7]. Other studies demonstrated that carbon dioxide insufflation induces local and systemic hypercarbia which affects metabolism and function of cells such as macrophages[2-5]. The capacity of macrophages to release TNF-α decreases upon carbon dioxide insufflation and the phagocytotic activity of macrophages is reduced as well during CO_2 exposure. In

a previous experimental study on the influence of pressure of carbon dioxide on tumor growth in rats, we found greater tumor growth with increasing insufflation pressures[1]. This observation suggests that tumor growth stimulating effects of CO_2 are pressure dependent. To avoid negative intrinsic effects of carbon dioxide, use of helium has been suggested. Helium is considered as an inert gas not interfering with metabolism or immunologic response. When compared to carbon dioxide in experimental studies, helium insufflation was associated with reduced tumor growth[7-9]. Air was not found to reduce tumor growth and supposedly does not influence acid-base equilibrium like carbon dioxide. Mikami et al. compared the influence of a pneumoperitoneum with air, N_2O and CO_2 at both 10 mm Hg and at 20 mm Hg on catecholamine plasma levels in pigs[10]. An increase in pressure to 20 mm Hg resulted in a significant increase of plasma catecholamine levels with all gases, whereas a pressure of 10 mm Hg had no effect. In a clinical study, Schilling et al. reported a significant decrease in microcirculatory splanchnic blood flow when the pressure of the carbon dioxide pneumoperitoneum increased from 10 to 15 mm Hg[11]. There was no significant difference of blood flow between 0 and 10 mm Hg pressure. Ishida and colleagues found an increased number of liver metastases after a 30 minute carbon dioxide pneumoperitoneum of 15 mm Hg compared to 5 and 10 mm Hg[12]. Samel and colleagues attached a special airtight window in the abdominal wall of rats which allowed observation by microscope of the blood flow in the jejunum during carbon dioxide pneumoperitoneum[13]. A decrease of jejunal blood flow was observed when insufflation pressure was raised from 10 to 15 mm Hg. Taking these observations into account, a threshold pressure possibly exists between 10 and 15 mm Hg. To investigate this assumption, an intermediate pressure group of 10 mm Hg was included in the current study. Tumor growth after gas insufflation at pressures of 4 and 10 mm Hg was similar while tumor growth was significantly increased after insufflating gas at 16 mm Hg pressure. This could support the hypothesis of a threshold pressure between 10 and 16 mm Hg, but further studies are required.

Although findings of experimental studies can not directly be extrapolated to daily clinical practice, we recommend performing laparoscopic oncologic surgery at the lowest insufflation pressure possible. In our experience, gasless laparoscopic surgery in patients is technically very demanding particularly in obese patients. Elevation of the anterior abdo-

minal wall requires forcefull retraction, and fail to suppress the viscera rendering proper exposure difficult. Therefore, it appears not possible to avoid gas insufflation of the abdominal cavity.

In this study, no difference of tumor growth were observed after either air or helium insufflation. This suggests that insufflation pressures per se are of greater importance than the type of gas.

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CHAPTER 6

PORT-SITE METASTASES.
IMPACT OF LOCAL TISSUE TRAUMA 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 tissue trauma and leakage of CO_2 along trocars ('chimney effect') in the development of port-site metastases.

Methods

Experiment I: Ten WAG rats had four 5-mm incisions in all abdominal quadrants. The incisions on the right side were crushed to induce tissue trauma. 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 min. Experiment II: Ten WAG rats had 5-mm incisions in the left and right abdominal upper quadrant. A 5-mm trocar was inserted in the incision in the left upper quadrant, and a 2-mm trocar was inserted in the incision in the right upper quadrant. 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: The median weight of tumor deposits at the trocar sites without induced tissue trauma was 22 mg. At the traumatic port-sites, median weight of tumor deposits was 316 mg ($p = 0.007$). Experiment II: The median weight of tumor deposits at the leaking trocar sites was 478 mg and at the control sites 153 mg ($p = 0.009$).

Conclusion

Tissue trauma at trocar sites and leakage of CO_2 along a trocar appear to promote implantation and growth of tumor cells at port-sites.

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 feasible and safe in malignant diseases as open surgery and, furthermore, is associated with less blood loss and reduced morbidity[1,2]. However, numerous case reports of port-site recurrences after diagnostic laparoscopy or laparoscopic resections in patiënts with malignancies have been published, which have caused great concern[3,4]. 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 suggests 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 tissue trauma. The traumatized trocar wound appears to be a good medium for implantation and growth of tumor cells because growth factors are abundantly present in traumatized tissue[5]. Another factor may be the leakage of CO_2 along a trocar, the so called 'chimney effect'[6]. 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 wound. This study was designed to determine the impact of both local tissue trauma at the trocar wounds and gas leakage along a trocar on tumor growth at the port-sites.

MATERIALS 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 h light-12 h dark), were fed a standard laboratory diet (Hope Farms, Woerden, The Netherlands), and had a 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

This study used CC-531, a 1,2-dimethylhydrazine-induced, weakly immunogenic, moderately differentiated colon adenocarcinoma, which is 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 (5,000 U/ml), 1 % streptomycin (5,000 U.ml) and 1 % L-glutamine (200 mmol). All supplements were obtained from Gibco (Paisley, UK). Before their use, cells were trypsinized (5 min, 37°C), centrifuged (5 min, 3,000 rpm), resuspended in RPMI-1640, and counted. Viability always exceeded 95 %. All tumor cells were injected in the rats within 5 hours after the cells were obtained.

Surgical Procedures

Operations were performed under general inhalation anesthesia using ether. After its abdomen 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. The laparoscopic equipment, provided by Karl Storz Endoscopes and Duffner, was adjusted to the rat. The laparoscope (diameter 4 mm), trocars (diameter 2 and 5 mm), and instruments were cleaned with 70 % alcohol before and after surgery.

Tissue Trauma 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 for 10 min to induce tissue trauma. 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, $5x10^5$ 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. Then CO_2 was insufflated through each trocar for 5 min. After 20 minutes all trocars were removed at the same time while the peritoneal cavity was still insufflated to prevent the trocars from touching 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 killed, and tumor deposits at all port-sites were precisely excised and weighed.

Gas leakage model

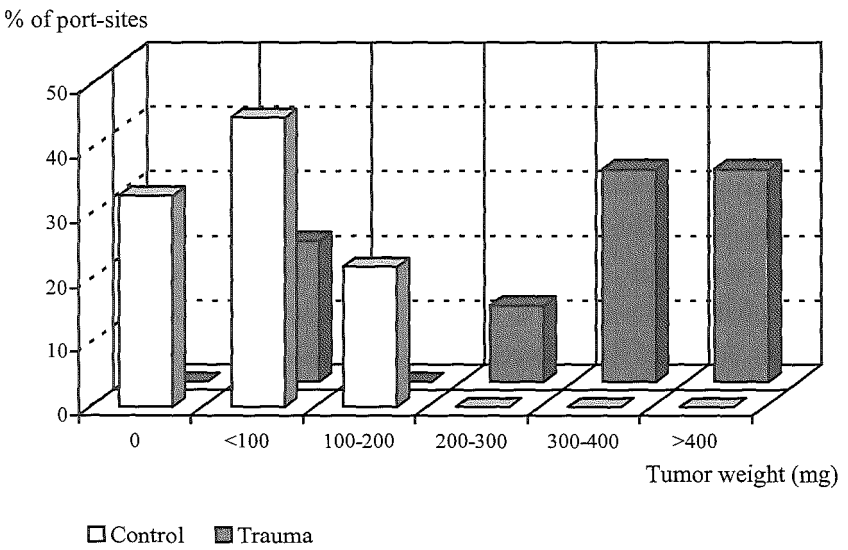
In 10 rats, a 5-mm trocar was inserted through a 5-mm incision in the left upper quadrant of the abdomen and secured with a purse-string suture (Vicryl® 3-0) to prevent leakage of CO_2. After creating a pneumoperitoneum with CO_2, absence of leakage was checked by the insufflator. Subsequently, the abdomen was desufflated. In the right upper quadrant of the abdomen, a 5-mm incision was made and a 2-mm trocar inserted. A pneumoperitoneum was established with CO_2 to a maximum pressure of 6 mmHg, and under laparoscopic vision, $5x10^5$ CC-531 tumor cells in RPMI-1640 medium were injected through the midline and equally distributed in the peritoneal cavity. Total amount of CO_2 leakage along the trocar in the right upper quadrant was set at 10 liters (15-20 min). 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 port-sites were resected and weighed. The Wilcoxon matched-pairs test was used to analyze the data for significant differences. Statistical significance was achieved at $p < 0.05$.

RESULTS

Tissue Trauma Model

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

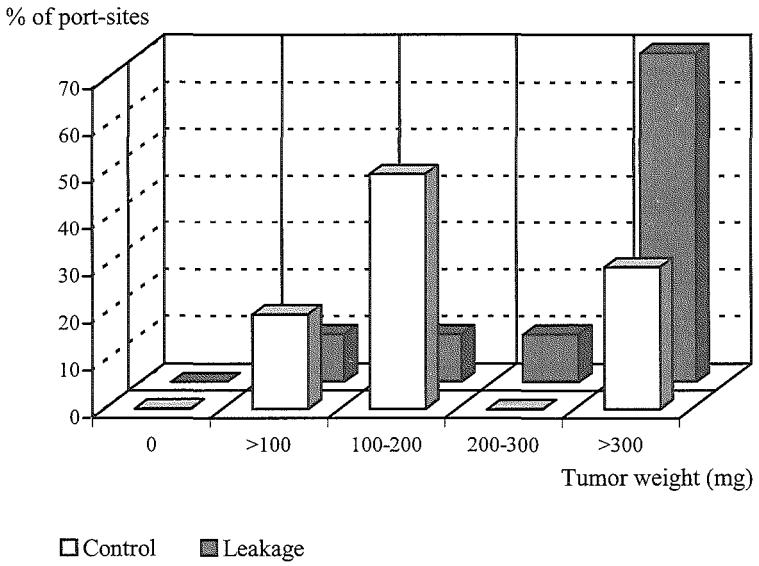
Figure 1. Distribution of tumor weight at traumatic port- and control sites



Gas Leakage Model

In all the rats, tumor take was found in the abdomen. Nine out of 10 rats had greater abdominal wall metastases at the port-sites with leakage along the trocar than at nonleaking port-sites. The median weight of tumor deposits at the leaking port-sites was 478.4 mg (48.0 - 968.0) and at the control port-sites 153.3 mg (41. 1- 459.1 mg). The median difference was 172.6 mg (37.3 - 927.9 mg), which is significant (p = 0.009) (Fig. 2).

Figure 2. Distribution of tumor weight at leaking port- and control sites



DISCUSSION

Tumor growth is enhanced by surgery, probably due to tumor manipulation and transient immunosuppression[7]. Laparoscopic surgery causes less tissue trauma than open surgery, and appears to be associated with less tumor growth and tumor take as shown in experimental studies[8,9]. However, tumor recurrence in abdominal wounds has been described after laparoscopy. The true incidence of wound recurrences after laparoscopy is unclear. At least 30 port-site metastases after laparoscopic colorectal operations have been reported, and incidences vary from 0-21 %[3,4]. Wexner et al.[4] estimated, on the basis of all presented and reported cases, that the incidence of port-site metastases is 4 %. In contrast, wound recurrence after conventional treatment of colorectal cancer is thought to be uncommon but possibly is underestimated. Hughes et al.[10] and Reilly et al.[11] reported incidences of abdominal wall recurrences after laparotomy for colorectal cancer of 0.8 % and 0.6 %, respectively. Others, however, report a more frequent occurence varying from 3.3 to 5.3 %[12]. The development of port-site metastases is probably multifactorial, and a number of possible mechanisms have been postulated. These mechanisms are discussed separately as follows.

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[13]. 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 port-site when the trocar is removed. This has been confirmed by Hewett et al,[14] who identified malignant cells in several washings of laparoscopic instruments and trocar wounds. Gas or fluid leaking along a trocar could act as a vehicle transporting tumor cells to the trocar wound, the so called 'chimney effect'[6]. In the gas leakage model we observed a significant rise in tumor weight at the leaking port-sites, suggesting such a mechanism. On the basis of this experiment, we are unable to differentiate aerosol leakage from leakage of fluid-containing tu-

mor cells as a causative factor in the development of wound metastases. Whelan et al.[15] failed to detect the presence of tumor cells containing aerosols in a high-pressure CO_2 environment. However, these autors 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 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*.

Tissue Trauma

It is well known that local factors influence the site and growth of metastases[16]. Murthy et al.[5] described the possibility of tumor cell entrapment in the clot formation in fresh wounds. Such clot formation could not only bind tumor cells, but also offer nutrition and a barrier against host defence 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[17]. Failing host defence 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 trauma model we observed a significantly higher tumor weight at the trocar sites that had been subjected to tissue trauma.

CO_2 influence

Some reports suggest a direct influence of CO_2 on tumor growth. In several experiments, Jacobi et al.[18] showed that insufflation of CO_2-promoted tumor growth compared with helium and control in a rat model. In our own laparoscopic experiments with rats, we found that tumor growth was significantly greater in a CO_2 insufflation group than in a gasless group[19]. However, Hubens et al.[20] failed to demonstrate any effect of CO_2 pneumoperitoneum on tumor cell implantation or growth. Therefore, the direct effects of CO_2 on tumor growth require further study.

A better understanding of the mechanisms contributing to port-site metastases is necessary to the development of ability to prevent them. Many authors already have advocated important preventive measures such as enlargement of extraction sites, protection of the extraction wound, the use of laparoscopic specimen bags, and less traumatic tissue handling. Leakage along trocar openings can be overcome simply with a purse-string suture around the trocar. Furthermore, it seems wise to prevent trocar wound trauma by not making the trocar incision too small. It can be argued that a purse-string suture itself induces tissue trauma and in that way can lead to enhancement of tumor adherence and growth. However, the fact that a significantly greater tumor growth was seen at the site of the leaking trocar than at the intact purse-string site, suggests that leakage contributes more to tumor growth than tissue trauma induced by a purse string. However, the use of balloon trocars can prevent the disadvantages of both leakage and tissue trauma. Clearly, the occurrence of tumor implantation and growth at port-sites is a complex process in which many factors are involved. Hopefully, further study of contributing factors will help us to overcome this serious problem in laparoscopic surgery for malignancy.

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CHAPTER 7

IRRIGATION OF PORT-SITES. PREVENTION OF PORT-SITE METASTASES?

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ABSTRACT

Introduction

Port-site metastases can occur when free viable tumor cells implant at trocar wounds. Irrigation of port-sites with cytotoxic agents has been suggested to prevent port-site metastases. The objective of this study is to assess whether tumor growth at port-sites can be reduced by irrigation of these port-sites.

Methods

WAG rats were insufflated with CO_2 for 20 min and $5x10^5$ CC-531 tumor cells were injected intra-peritoneally. Port-sites were irrigated after completion of the pneumoperitoneum with povidone-iodine, a mixture of taurolidine and heparin or sodium chloride. Controls did not undergo any irrigation of port-sites. In experiment I, all 16 rats had all 4 irrigation modalities. In experiment II, four groups of 20 rats had one type of irrigation on two trocar wounds. Tumor growth was evaluated 4 weeks after the procedure.

Results

No difference in tumor growth at trocar wounds was found between any type of irrigation and controls in both experiments

Conclusion

In this experimental model, no beneficial or adverse effects of irrigation of port-sites could be shown.

INTRODUCTION

The occurrence of port-site metastases after laparoscopic resection of colon cancer questioned the oncological safety of laparoscopic surgery. Some proclaimed that laparoscopy was not suitable for curative oncologic surgery while others advocated scientific evaluation in randomized trials. It was recognized that proper surgical technique was important to prevent port-site metastases. In addition, special measures were developed to prevent port-site metastases[1-4]. Irrigation of port-sites at the end of surgery has been suggested as prevention of port-site metastases. The objective of this experimental study was to assess the efficacy of local irrigation of port-sites with various solutions to prevent local tumor growth.

MATERIALS & METHODS

Animals

Male rats of the inbred WAG/Rij strain (Wistar Agouti / Rijswijk), weighing 250-300 g and bred under specific pathogen-free conditions (Harlan, Austerlitz, The Netherlands), were kept under the following conditions: temperature of 20-24 °C, relative air humidity of 50-60 % and a 12 hours light-12 hours dark cycle. The animals had free access to water and food (Hope Farms, Woerden, The Netherlands) before and after surgery. The protocol was approved of by the Committee on Animal Research of Erasmus University, Rotterdam, The Netherlands.

Tumor

CC-531 is a moderately differentiated colonic adenocarcinoma, chemically (1,2-dimethylhydrazine) induced in the WAG strain. This tumor is tranplantable in syngeneic WAG rats and weakly immunogenic as determined by the immunization challenge method described by Prehn and Main[5]. The tumor was kept *in vitro* in RPMI 1640 medium with 5 % fetal calf serum (virus and mycoplasm screened), 1 % penicillin (5000 U/mL), 1% streptomycin (5000 U/mL) and 1 % L-glutamine (200 mmol/L). All supplements were

obtained from Gibco, Paisley, UK. For preparation of the tumor cell suspension, cells were trypsinized (5 min. at 37 °C), centrifuged (5 min 3000 rpm), resuspended in RPMI 1640 and counted. Viability of more than 95 % of cells was checked by trypan blue staining (0.3 % in 0.9 % sodium chloride solution). A suspension of 5 10^5 cells per mL was prepared and stored on ice until injection in the rats.

Procedures

A closed box which could be connected to ether-saturated air was used for induction of anesthesia in rats. Ether anesthesia was maintained during the experiment with an ether-saturated plug of cotton in a tube placed over the snout of the rats. An automatic insufflator (Van Straaten Medical, Nieuwegein, The Netherlands) established a pneumoperitoneum with room temperature CO_2. Disposable 5 mm trocars (Surgiport, US Surgical, Norwalk, CT, USA) were shortened for use in rats.

Three irrigation solutions were used in both experiments. Sodium chloride 0.9 %, povidone-iodine 1 % and a mixture of taurolidine 0.5 % with heparin (100 U/mL). Taurolidine (Taurolin, Hoechst, Germany) is a derivative of taurine, which is one of the essential amino acids[6]. A small cotton-wool swab stick, like an ear cleaning stick, was dipped in one of the solutions and turned in the trocar wound.

Experiment I

Sixteen rats were used in the first experiment. In each rat, 4 shortened five mm trocars were introduced in each abdominal quadrant and fixed with a transcutaneous purse string. After insufflation of the abdomens to a pressure of 6 mm Hg, $5x10^5$ tumor cells were injected intra-peritoneally through the midline of the abdomen. In 20 minutes, 5 liters of CO_2 were allowed to flow from the insufflation port in the right upper quadrant through the abdomen to the venting port in the left lower quadrant. At the end of this period, the ports were extracted and the wounds were irrigated according to the following protocol. Each of the four wounds per rat received a different treatment. One wound was irrigated with a swap stick with sodium chloride 0.9 %, one with a swab stick with povidone-iodine 1 % and another with a swab stick with taurolidine 0.5 % with heparin 100 U/mL. One wound was left untreated as a control. In the 16 rats, each type of irrigation was applied 4

times in each quadrant to preclude a bias by anatomical site or by insufflation or venting port-site. After treatment, the wounds were elevated to avoid contact with the abdominal contents and closed with a single atraumatic ethilon 3-0 (Ethicon, Summerville, NJ, USA) through all layers (skin, muscle and peritoneum). Four weeks later all rats were sacrificed and tumor deposits at each port-site were resected and weighed. The investigators were blinded for the previous treatment of the port-sites.

Experiment II

Eighty rats were used in the second experiment. They were randomly allocated to 4 treatment groups: Sodium chloride 0.9 %, povidone-iodine 1 %, taurolidine 0.5 % with heparin 100 U/mL or no treatment. In each rat, 2 shortened five mm trocars were introduced (one in the right half and one in the left half of the abdomen) and fixed with a transcutaneous purse string. After insufflation of the abdomens to a pressure of 6 mm Hg, 5×10^5 tumor cells were injected through the midline. In 20 minutes, 5 liters of CO_2 were allowed to flow from the insufflation port in the right abdomen through the abdominal cavity to the venting port in the left abdomen. Subsequently the trocars were extracted and the wounds were treated according to the randomization of the animal (so both wounds had the same treatment in one animal). Technique of wound treatment and closure were identical to experiment I. Four weeks later all rats were killed and tumor deposits at each port-site were resected and weighed. The investigators were blinded for the previous treatment of the port-sites. Data were analyzed using analysis of variance (ANOVA) and results were considered statistically significant at $p < 0.05$.

RESULTS

Experiment I

The variation of tumor mass at the port-sites in each treatment group (n = 16) was substantial, as shown by large standard deviations. In table 1, mean and median mass of tumor at the port-site, standard deviation, range and total mass of tumor at the 16 port-sites of one treatment group are shown.

Total weight of tumor at port-sites per animal ranged from 11 to 6209 mg. Statistically, no significant differences were found.

Table 1. Experiment 1: Port-site tumor mass per treatment after 4 weeks

Port-site treatment	N	Tumor mass at port-sites in mg				
		Mean	Median	S.D.	Range	Total
Sodium chloride	16	387mg	304mg	± 346mg	0-1166mg	6198mg
Povidone-iodine	16	541mg	160mg	± 915mg	0-3190mg	8659mg
Taurolin / Heparin	16	840mg	492mg	± 931mg	0-3104mg	13442mg
No treatment	16	558mg	198mg	± 645mg	0-2112mg	8935mg

Experiment II

In the rats of experiment II, again a substantial range of tumor growth was found. As each rat received only one kind of port-site treatment, the mass of tumor at both port-sites were added up. In table 2 the mean mass of tumor at the port-sites in each animal, the median, the standard deviation, the range and the total mass of tumor in 20 animals in each treatment group are shown. Statistically, no significant differences were found.

Table 2. Experiment 2: Port-site tumor mass per treatment after 4 weeks

Port-site treatment	N	Tumor weight at port-sites in mg				
		Mean	Median	S.D.	Range	Total
Sodium chloride	20	201mg	27mg	±361mg	0-1360mg	4021mg
Povidone-iodine	20	167mg	2mg	±314mg	0-1047mg	3346mg
Taurolin / Heparin	20	178mg	45mg	±408mg	0-1814mg	3562mg
No treatment	20	175mg	6mg	±557mg	0-2518mg	3494mg

DISCUSSION

The pathogenesis of port-site metastases raises many questions, but there is consensus on the fact that implantation of free intra-abdominal viable tumor cells in the wounds of trocars is a pivotal factor. Animal studies with radiolabelled tumor cells in pigs revealed that laparoscopic instruments can carry tumor cells to the port-sites, initiating tumor growth at these sites[7-9]. Another experimental study showed less port-site recurrences when both instruments and port-sites were cleaned frequently during surgery[2]. A sensible and straightforward concept to diminish intraperitoneal and abdominal wall recurrence are peritoneal washings with cytotoxic agents to reduce the number of viable free tumor cells. Studies in rats showed a reduction of intraperitoneal tumor growth and port-site metastases after peritoneal washings with povidone-iodine[6,10,11]. In a rat study investigating local recurrence, peritoneal washings with sodium hypochlorite and again povidone-iodine significantly reduced tumor growth[12]. An intraperitoneal mixture of taurolidine and heparin successfully inhibited tumor growth and/or implantation in another laparoscopy rat study by Jacobi and colleagues[6]. One of the drawbacks of cytotoxic washings is, as outlined by Jacobi et al, the possible damage to peritoneal macrophages and the peritoneum itself, which have an important defensive function against tumor cell implanta-

tion[6,13,14]. Based on the findings mentioned above, we performed two experiments in which we compared the efficacy of several agents when used locally to irrigate the port-sites without a complete peritoneal washing. We used two solutions with different anti tumor-cell mechanisms because they were shown to be effective in similar animal experiments, and a neutral third solution (sodium chloride) to rule out the mechanical component of cleaning the port-site with a swab-stick. In addition to this, each experiment included non treated trocar wounds as a control group. Povidone-iodine is a cytotoxic agent that reduces the number of free viable tumor cells. When used as a lavage in animal experiments, it reduced tumor growth[6,10,11]. Heparin inhibits tumor cell adherence. One of the factors involved in tumor recurrence is adherence of free tumor cells to specific substances. When peritoneum is damaged, the extracellular matrix is exposed and this matrix contains proteins and collagens to which receptors of cells can bind. This was experimentally shown by blocking parts in the extracellular matrix, which resulted in diminished adherence of tumor cells[15,16]. Heparin has been shown to reduce adherence of tumor cells and bacteria to urothelium or peritoneum, possibly by binding to fibronectin[10,17-20]. Taurolin has both an inhibitory and an anti-adhesive effect. Taurolin inhibits production of interleukin-1 (IL-1) by peripheral blood mononuclear cells[21]. Interleukin-1 promotes cell proliferation directly and indirectly by stimulation of other growth factors[22] and its inhibition reduces tumor cell growth[10]. Taurolin was also demonstrated to be anti-adherent, inhibiting tumor cell growth in a similar way as heparin[23]. Jacobi and colleagues found a significant reduction of peritoneal tumor growth after lavage with a mixture of heparin and taurolin[6,10]. The experimental model involving the WAG/Rij rat with the syngeneic CC-531 tumor cell line is well established at our laboratory and several studies proved its reliability and usefulness in experimental laparoscopic research[24,25]. In the first experiment we chose for a design in which all treatments were applied in one animal. The benefit of such a design is the reduction of inter animal differences, thereby reducing standard deviations and clarifying statistical differences. Unfortunately, standard deviations were great and no statistical conclusion could be drawn. As we could not identify a clear cause for the large range of results, we decided to repeat the experiment with a simplified design. We used just two ports and only one treatment per animal. Because inter animal differences could be greater we decided to study more animals. In the first experiments

we had 16 measuring points per treatment (one port per animal) and in the second 40 per treatment (two ports in 20 animals). In this second experiment again, no differences between groups could be found. Because of a rather wide range of tumor growth, the process of preparing the tumor cell solution was reviewed. No abnormalities of tumor cell viability or concentration were found. Local application of the irrigating agents as described in our experiments had no enduring anti tumor effect. The agents may have been diluted and washed out of the wound shortly after closure of the wounds, in spite of the fact that great care was taken to close all layers before the wound could have contact with the abdominal contents. On the other hand, local cell destruction by povidone-iodine could have caused increased local trauma and could hypothetically have stimulated local tumor growth. In these experiments however, no stimulating effect on tumor growth of any test agent was found. Hoffstetter and colleagues[26] performed a similar rat study in which they did find a reduced recurrence rate at the port-site wounds after treatment with povidone, but they only scored tumor masses of which more than 50 % of the volume was actually in the wound, whereas we weighed every tumor growth at the port-site. Esh-raghi and colleagues[27] found less port-site tumors after wound irrigation with 5-FU in a rat model. Differences in animal model and tumor cell line, definition of port-site recurrence, system of scoring recurrences and of course the irrigating agent itself can all be responsible for conflicting results after local application of tumoricidal agents. The benefit of irrigation of port-sites in oncologic surgery remains unclear and other experimental models or clinical trials are needed to evaluate the efficacy of port-site irrigation.

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CHAPTER 8

LESS TUMOR TAKE AFTER ULTRASONIC DISSECTION? AN EXPERIMENTAL STUDY.

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ABSTRACT

Background

Experimental studies showed a correlation between degree of peritoneal trauma and tumor take. Since ultrasonic surgery is supposedly less traumatic than electrosurgery, the impact of ultrasonic dissection on tumor take was investigated in an animal model.

Methods

Sixteen rats were randomized to either ultrasonic or diathermic surgery. Bilateral incisions were made in the parietal peritoneum by either ultrasonic or diathermic devices. Subsequently, a syngeneic tumor cell suspension was injected intraperitonally and tumor take was evaluated after two weeks.

Results

Tumor take at the peritoneal incision was significantly greater ($p = 0.001$) in the diathermic group (3313 ± 125 mg) than in the ultrasonic group (1554 ± 81 mg).

Conclusion

Ultrasonic dissection induces less tumor take than electrosurgery in an experimental setting. Further studies are necessary to evaluate the role of ultrasonic surgery.

INTRODUCTION

Ultrasonic instruments became available in the early eighty's for surgical dissection. The basis of ultrasonic dissection is fragmentation and disruption of tissue due to high frequency longitudinal vibration of the tip of the instrument[1]. The first ultrasonic instruments had a relatively low frequency of, for instance 23 kHz in the Cavitron Ultrasonic Surgical Aspirator (CUSA, Valleylab, Boulder, CO, USA). These instruments caused fragmentation of cells only and spared collagen-rich tissue like nerves, vessels or bile ducts. This quality provided to be effective and safe in dissection of parenchymatous organs such as the liver. After fragmentation of the cells, remaining blood vessels and bile ducts could be coagulated, clipped or ligated. Ultrasonic instruments that apply a relatively low frequency serve therefore only to safely expose the skeleton of vessels and ducts. In a later generation of ultrasonic instruments, a higher frequency of 55.5 kHz was applied. The higher frequency resulted in a greater energy transfer to the tissue and thereby allowed transsection of collagenous tissue. In addition, denaturation of proteins and local heat generation due to friction provide the possibility to transect and seal small and intermediate size vessels and ducts[1,2]. These high frequency ultrasonic instruments, like the Harmonic scalpel, enable both bloodless dissection of tissues and coagulation of vessels, and are therefore an alternative to electrosurgery[3,4].

Ultrasonic mechanical energy appears to have some important benefits to monopolar electricity in surgery, particularly with regard to safety. Because the temperature at the tip of the instrument remains relatively low (< 80°C), there is no smoke or charring of tissue that could obscure the surgical field[1]. The low temperature also reduces the risk of thermal damage to viscera when the instrument inadvertently touches other tissues. For comparison, the heat generated by diathermy may rise to 500°C[5]. Another specific risk of monopolar electrosurgery is the fact that the electrical current can take an unexpected pathway to the ground plate, causing inadvertent and unrecognized thermal injury to tissues[6]. In electrosurgery, proper hemostasis is sometimes not possible because tissue sticks to the instrument after coagulation and a vessel can be torn open while retracting the instrument[4]. As the higher frequency vibration of the blade impairs adherence of tissue, this problem is rarely encountered in the harmonic scalpel.

Hambley et al. studied histopathologic characteristics and healing of skin incisions in pigs made by a conventional scalpel, the harmonic scalpel, a diathermic instrument and carbon dioxide laser[7]. To measure histopathologic changes, fresh wounds were excised for microscopical assessment of epidermal destruction and collagen denaturation lateral to both wound edges. Diathermia and carbon dioxide laser were significantly inferior to the harmonic scalpel in terms of secondary tissue trauma and speed of wound healing. This was attributed to considerable generation of heat by electrosurgery and carbon dioxide laser and dispersal of heat into the surrounding tissue. Tissue trauma appears to play an important role in implantation and growth of tumor cells. The relation between peritoneal trauma and tumor growth was, amongst others, demonstrated by Eggermont et al.[8]. They found that, after intraperitoneal injection of a tumor cell suspension, a laparatomy in mice resulted in enhanced growth of tumor compared to incision of the skin on the back of the animal. Given these findings, we performed an experimental study to assess tumor take after either ultrasonic surgery or electrosurgery.

MATERIALS AND METHODS

Animals

Sixteen male rats of the inbred WAG/Rij strain, weighing 200-250 g and bred under specific pathogen-free conditions (Harlan, Austerlitz, The Netherlands), were used. At our laboratory, they were kept under the following conditions: temperature of 20-24°C, relative air humidity of 50-60 % and a 12 hours light-12 hours dark cycle. The animals had free access to water and food (Hope Farms, Woerden, The Netherlands) before and after surgery. The protocol was approved of by the Committee on Animal Research of Erasmus University, Rotterdam, The Netherlands.

Tumor

CC-531 is a moderately differentiated colonic adenocarcinoma, chemically (1,2-dimethylhydrazine) induced in the WAG strain. This tumor is transplantable in syngeneic WAG rats and weakly immunogenic as determined by the immunization challenge me-

thod described by Prehn and Main. The tumor was kept *in vitro* in RPMI 1640 medium with 15 % fetal calf serum (virus and mycoplasm screened), 1 % penicillin (5000 U/ml), 1 % streptomycin (5000 U/ml) and 1 % L-glutamine (200 mmol/L). All supplements were obtained from Gibco, Paisley, UK. For preparation of the tumor cell suspension, cells were trypsinized (5 min. at 37°C), centrifuged (5 min 3000 rpm), resuspended in RPMI 1640 and counted. Viability of more than 95 % of cells was checked by tryptan blue staining (0.3 % in 0.9 % sodium chloride solution). A suspension of 0.5 x 10^6 cells per ml was prepared and stored on ice until injection in the rats.

Instruments

A closed box which could be connected to ether-saturated air was used for induction of anesthesia in rats. Ether anesthesia was maintained during the experiment with an ether-saturated plug of cotton in a tube placed over the snout of the rats. An Erbe ICC 50 (Elektromedizin, Tübingen, Germany) transformer with a 5 mm hook instrument for laparoscopic surgery was used to inflict the diathermic lesions. Current was set at 40 in the forced mode. Ultrasonic lesions were made with the dissection hook for open surgery of the UltraCision (Ethicon Endo-Surgery Inc, Cincinnati, OH, USA), set at level 6 in the cutting mode.

Procedure

Rats were numbered and subsequently randomized to either the ultrasonic group or the diathermic group. After induction of anesthesia, the rats were placed in supine position and their abdomens were opened by a 5 cm midline laparotomy. The abdominal wall was everted and at both sides, a 2.5 cm longitudinal incision was made through the parietal peritoneum and the inner muscular layer in the following way. A small hole just through the inner muscular layer was made by pressing either the activated diathermic or ultrasonic hook against it. Subsequently, the inner muscular layer was lifted with the hook of the instrument and the muscular layer was opened by tearing the activated instrument through the tissue. After creating both lesions, 500.000 tumor cells in 1 ml were injected in the abdomen and the abdominal wall was closed in one layer. The rats were sacrificed two weeks later. Tumor take at the abdominal lesions was determined by dissecting and

weighing the tumors. Tumor take at other intraperitoneal sites was assessed semi quantitatively as described hereafter. Tumor take was assessed at six intra-abdominal locations: the right kidney, the left kidney, the mesentery, the liver, the retroperitoneal surface and scrotal fat. Two independent blinded observers estimated tumor take at each location according to the following scoring system: 0: no tumor take, 1: minor tumor take, 2: moderate tumor take, and 3: extensive tumor take. In case of disagreement between the observers, the score was middled. No definition of minor, moderate or extensive tumor take can be given, because the difference in peritoneal surface of the locations and incomparability of tumor take at each location. In general, a score of 3 represented many lesions with a diameter of > 2 mm or a tumor mass with a diameter of > 5 mm. Results were analyzed with Kruskal-Wallis tests.

RESULTS

With the diathermic hook, more time and force was needed to induce the lesions. After completing the lesions, the borders of the diathermic wounds were whitened by heat up to 3 mm from the wound and they showed carbonization. The borders of the ultrasonic wounds showed only whitening up to 1 mm from the wound. The mean weight of dissected tumors from both abdominal wall lesions per animal was 1554 mg (± 81 mg) in the ultrasonic surgery group and 3313 mg (± 125 mg) in the diathermic group. Statistical analysis revealed a significant difference ($p = 0.001$, Table). Tumor growth at the midline incisions was minute in both groups. The mean tumor score at the liver was significantly lower in the ultrasonic group than in the diathermic group ($p = 0.036$, Table). The mean scores of tumor growth at other sites and the total mean scores of intraperitoneal tumor load were not significantly different. No animals dropped out of the study because of mortality or other complications.

Table. Mean tumor score per visceral site and tumor mass at parietal incisions.

Visceral site	Ultrasonic	Diathermic	p
Kidney R	1.6 (± 0.2)	1.0 (± 0.3)	ns
Kidney L	1.3 (± 0.2)	0.8 (± 0.3)	ns
Mesentery	2.1 (± 0.4)	2.1 (± 0.2)	ns
Liver	1.4 (± 0.2)	2.0 (± 0.2)	0.036
Retroperitoneum	2.4 (± 0.3)	2.0 (± 0.3)	ns
Scrotal fat	1.3 (± 0.3)	1.5 (± 0.2)	ns
Total	100 (± 1.0)	9.4 (± 1.2)	ns
Mass at parietal incisions	1554 mg (± 81)	3313 mg (± 125)	0.001

DISCUSSION

Ultrasonic surgery was associated in this study with significantly less local tumor take and growth than electrosurgery and we hypothesize that this difference resulted from a difference in peritoneal trauma. Although we did not exactly quantify the extent of collateral surgical trauma during the experiment, a considerable difference in direct tissue injury between the two instruments was observed macroscopically. During evaluation of tumor load it appeared that one rat in the diathermic group had a large tumor deposit in the hepatic hilus. This tumor lump augmented the mean score just enough to get a significant difference (Table). However, this side had not been in contact with the abdominal wall lesions and it seems unlikely that this difference in tumor take was caused by the experimental treatment. Like other studies, the results and observations of this experiment reflect the idea that intraperitoneal tumor growth is influenced by the degree of peritoneal trauma. Bouvy et al. demonstrated that, after intraperitoneal injection of a tumor cell sus-

pension, a laparoscopic assisted bowel resection in rats resulted in less tumor take than a bowel resection via a full laparotomy[9]. Van den Tol et al. mimicked a difference in peritoneal trauma by rubbing the uterus horns of female rats with normal surgical gauze or soft gauze used in the electronics industry and subsequently injected a tumor cell suspension[10]. The uterus horns rubbed with the soft gauze showed significantly less tumor take. These findings shed light on a new aspect of cancer surgery. Locoregional recurrence after colorectal resection with curative intent for carcinoma is seen up to 20 % and surgeons are trying to reduce this complication with different strategies[11,12]. The results of our experiment and the aforementioned studies suggest that the extent of surgical trauma may also influence local recurrence in a clinical situation. Likewise, these results show that the use of an ultrasonic instead of a diathermic instrument in cancer surgery could potentially reduce locoregional recurrences. Another possible factor influencing tumor recurrence is per-operative dispersal of viable tumor cells. When activated and in contact with tissue, the harmonic scalpel produces a visible 'cloud' of small particles, which could contain viable tumor cells. Nduka et al. investigated this possibility by ultrasonically incising tumors in prepared rats and aspirating the cloud[13]. Microscopic inspection using trypan blue dye exclusion did not show any viable cell and there was no growth in cell culture. Thus, the risk of dispersal of viable tumor cells during ultrasonic surgery seems negligible. Laparoscopic surgery demands dissection with minimal bloodloss and a reletively easy control of blood vessels. Therefore, diathermic dissection and electrocoagulation is probably used more frequently and longer in a laparoscopic than in an open procedure, potentially causing more surgical trauma. Consequently, laparoscopical surgery benefits more than conventional surgery from an instrument that combines dissection and coagulation qualities with less collateral surgical trauma. Like mentioned before, the harmonic scalpel produces a cloud of particles when activated. Unlike smoke produced with electrocoagulation, this matter precipitates quickly and does not hamper visibility. Dangers of monopolar electrosurgery are more pronounced in laparoscopic procedures because of the restricted view of the scope and relatively small working space. These conditions increase the risk of unrecognized injury to viscera due to unintended direct or indirect contact with the activated instrument[6]. Reduction of operating time is another benefit of laparoscopic application of ultrasonic surgery. Coagulation of blood vessels is easier and

faster than with diathermia and larger blood vessels can safely be coagulated, reducing the need for hemostatic clips and accompanying change of instruments. Changing instruments is time consuming, disturbs the visual contact with the operating area and often results in loss of pneumoperitoneum pressure. In conventional surgery, freedom of movement and unrestricted use of different techniques will often enable faster surgery than when a surgeon confines himself to an ultrasonic instrument. The harmonic scalpel has several benefits over electrosurgery that become especially apparent in laparoscopic procedures: operating time reduction, less hampering of visibility and no inadvertent thermal or electrical injury. In addition to this, it produces less collateral surgical trauma than electrosurgery, resulting in less tumor take in an experimental setting. In conclusion, the use of ultrasonic instruments instead of electrocoagulation in oncologic surgery deserves serious consideration. Especially laparoscopic oncologic procedures may benefit from ultrasonic surgery, but further studies are needed to confirm this.

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CHAPTER 9

GENERAL DISCUSSION

GENERAL DISCUSSION

The introduction of laparoscopic surgery for colon cancer was followed by growing concern about the oncological safety of this new surgical approach. The technical difficulty and the lack of tactile senses in laparoscopic surgery caused concern about the extent of resection margins and lymph node harvest. Simultaneously, case reports on metastatic tumors in trocar (or port) wounds appeared. In early small series, the incidence of these trocar wound or port-site metastases was alarmingly high. Most of these port-site metastases occurred in patients with known dissiminated disease, but in some it was the first or the only symptom of dissimination (chapter 2). These reports, though few in number, left the impression that port-site metastases were a threatening new phenomenon related to laparoscopic surgery. It was an incentive to prudence and both clinical and laboratory studies were designed with special attention for port-site metastases. The first reported series of laparoscopically operated patients showed comparable resection margins and lymph node harvest as open surgery, but had only limited follow up[1,2]. As more clinical data became available, the incidence of port-site metastases dropped considerably. Incidences appeared to be comparable to those known in conventional surgery (chapter 2). Reasons for this decline of incidence of port-site metastases were probably the fact that the learning curve was passed by many contributing surgeons and better quality of reported studies. These studies, though seldom randomized, included more patients with prospectively collected data and were less biased by patient selection. Initial reports often included many patients for palliative treatment, who subsequently developed port-site metastases when dissimination progressed.

Experimental studies suggest an oncologic benefit of laparoscopic surgery over conventional surgery. Open surgery results in more local and systemic tumor growth in animal models than laparoscopic surgery[3,4] and immune responses are better preserved after laparoscopic procedures compared to conventional procedures [5-7].
The benefit of laparoscopic surgery over conventional surgery is related to reduced tissue trauma and 'surgical stress' inflicted by the laparoscopic technique. This reduction is ma-

croscopically obvious when comparing a laparotomy wound to several trocar wounds. In both experimental and clinical studies, serological markers used to monitor surgical stress showed attenuated responses in laparoscopically treated individuals[8-10]. Less obvious is the reduction of trauma to the peritoneal surface. Preservation of an intact peritoneal surface has important consequences for tumor adherence and immunology. In several experiments it was shown that peritoneal trauma results in more loco-regional tumor adhesion and growth[11-14]. In conventional abdominal surgery, small bowel and other organs are packed away with dry abrasive gauzes behind tight placed retractor blades. In addition to this, the operated organ itself is often manipulated with gauze to prevent slipping away. These actions result in serious damage and ablation of a large area of peritoneum[12] and prolonged local ischaemia of organs by pressure of retractor blades. In laparoscopic surgery, no gauzes enter the abdomen and organs are usually retracted by a solitary instrument, which probably reduces peritoneal damage. A movement towards minimally invasive and minimally traumatic surgery is also aided by development of new devices like ultrasonic dissection instruments that combine effective hemostasis with minimal collateral tissue damage (chapter 8). In addition to mechanical trauma, the peritoneal surface is damaged by exposure to air or insufflation gas. In scanning electron microscopy studies, alterations of the integrity of the peritoneal surface have been described as a result of exposure to air in gasless laparoscopy and to insufflation with carbon dioxide[15,16]. The study of Hazebroek et al.[15] sustains the conclusion that the detrimental effect of gas exposure to peritoneum is similar in laparoscopic and conventional surgery, as the exposure of peritoneum to air in gasless laparoscopy is comparable to conventional surgery. In contrast to conventional surgery however, the enclosed operating area in laparoscopic surgery offers the possibility of humidifying and warming insufflation gas, which may reduce desiccation and alterations of the peritoneal layer.

In spite of oncologic benefits of laparoscopic over conventional surgery in experimental studies and a decline in reported incidence of port-site metastases, these recurrencesremain an actual and intriguing problem. Two illustrating reports of puzzling behaviour of port-site metastases describe recurrences from occult colon cancers in trocar wounds of laparoscopic cholecystectomies that were performed *before* diagnosis of the colon tu-

mors and subsequent colon resections, while no recurrences were found in the laparotomy scars of the colectomies[17,18]. Some characteristics of laparoscopy apparently facilitate implantation of tumor cells at trocar wounds. In laparoscopic surgery, instruments are in close contact to their entry point in the abdominal wall. Movement of dissection instruments in and out of trocars transports free tumor cells from the tumor bearing organ to the abdominal wall[19]. Ischaemia and necrosis of wounds around trocars may weaken local anti-tumor defenses and may cause local release of cytokines, thus favoring implantation of tumor cells[20]. Gas leaking along trocars, the 'chimney effect', may carry a fluid film or fluid drops containing free tumor cells to the trocar wound[20] (chapter 6). Local acidification of tissues by carbon dioxide insufflation may blunt local immune responses and reduce local tumor cell destruction[21,22]. All these mechanisms potentially contribute to port-site metastases development, but the weight of the individual factors in the etiology of these metastases remains unclear.

The appearance of port-site metastases and the study of their pathogenesis gave rise to several peroperative measures in order to prevent these metastases[23]. Most of these measures are meant to prevent implantation of free viable tumor cells in trocar wounds. Careful placement and (suture) fixation of trocars prevents a chimney effect (chapter 6) and precludes trocar dislodgment resulting in fluid leakage in the wound. Careful suction, deflation solely through trocars and closing of peritoneum could theoretically reduce postoperative leakage of fluid containing free tumor cells into trocar wounds[23]. Postoperative cleaning of port-sites with cytotoxic agents was not effective in an experimental setting in this thesis (chapter 7). Protection of the wound with plastic draping during specimen excorporation seems a very important measure to prevent direct tumor cell implantation and is widely advised[23,24], especially in trials concerning laparoscopic colon surgery for cancer. A critical appraisal of the pneumoperitoneum offers another way to prevent port-site metatases. Type of gas and applied intraabdominal pressures can both influence tumor cells and local tissue conditions of the patient. In this thesis, a higher intra-abdominal pressure with different gases was found to result in more tumor growth in rodents (chapter 4 and 5). Many of the peroperative measures to prevent port-site metatases seem sensible and some are supported by laboratory studies, but their significance in humans is

difficult to assess. The evaluation of these measures in clinical randomized trials is thwarted by the low incidence of port-site metatases and ethical objections.

Although laboratory research revealed some essential steps of the pathogenesis of port-site metastasis in a relatively short time, it seldom provides undisputable conclusions that can directly be extrapolated to laparoscopic surgery in cancer patients. Laboratory studies have obvious important benefits over clinical research: investigated factors can be isolated, standardization of circumstances and procedures is relatively easy, results come fast and laboratory work avoids putting patients at risk. A major disadvantage is that simplification of clinical reality to a reproducible animal model risks to be non-representative. Examples of these simplifications in many laparoscopy studies are the fact that most animals were not mechanically ventilated during surgery, which influences hypercapnia[25,26], or the high number of intraperitoneally injected tumor cells in animal experiments compared to the number of free intraperitoneal tumor cells in patients without disseminated cancer (chapter 3)[27,28]. Another problem is the comparability of similar laboratory studies. Differences in type or strain of animal, differences in tumor cell line and differences in procedure can all be responsible for dissenting results of studies with the same objective. Notwithstanding the fact that laboratory work is indispensable for the introduction of new techniques, the interpretation of results is difficult. Therefore, clinical implications of theories based on laboratory work can only emerge from large randomized trials. Several multicenter randomized trials comparing conventional open surgery with laparoscopic surgery for colon cancer have started in the second half of the ninety's and have completed inclusion of patients[18,29-31]. Results of these studies are expected in the end of 2004.

The future of laparoscopic colon surgery depends on the long-term survival and recurrence outcome of the above mentioned randomized trials. A single centre randomized trial including 219 patients with a median follow up of 43 months showed faster recovery in patients who had a laparoscopic resection for colon cancer[32]. Interestingly, this trial showed improved survival in the laparoscopically treated group. The Laparoscopic Colectomy Trial of the Clinical Outcomes of Surgical Therapy (COST) Study Group included 863 patients with a median follow up of 53 months[33]. This trial also showed impro-

ved short term outcome in the laparoscopic group, but cancer related survival was identical in laparoscopic and open operated patients. These trials indicate that laparoscopic resection of colon cancer is safe and oncologically justified. If other large multi center randomized trials[18,29,31] confirm identical survival and recurrence rates after conventional and laparoscopic colon resection for cancer, quality of life and cost studies will determine the future role of laparoscopic oncologic colon surgery. However, if the above mentioned or future trials show an oncological benefit on the side of laparoscopic surgery, like the study of Lacy et al.[32], a faster general acceptance and application of laparoscopic surgery for colon cancer should be expected. Another major factor in the acceptance of laparoscopic oncologic surgery is the technical difficulty and resulting learning curve. Currently, surgical residents and young surgeons have more experience with miscellaneous laparoscopic procedures than five or ten years ago and this stimulates laparoscopic surgery in general. In addition to this, new techniques and instruments may facilitate laparoscopic colon surgery. Better optics, cameras and screens improve the view of the operating area and eye-hand coordination. Instruments that effectively combine dissection, coagulation, flushing and suction will importantly speed up laparoscopic procedures and enhance laparoscopic surgical possibilities. Notwithstanding the fast spread of laparoscopic experience and development of techniques, laparoscopic colon surgery may not be in the scope of every general or gastro-intestinal surgeon or surgical department and remain confined to specialized surgeons and centres for years to come.

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CHAPTER 10

SUMMARY & CONCLUSIONS

SAMENVATTING & CONCLUSIES

SUMMARY

Chapter 1

A short survey of technique and history of laparoscopic surgery is presented. Subsequently, the ethical and practical problems involved in introducing a new technique like laparoscopic surgery in oncology are discussed. The occurrence and possible causes of port-site metastases are discussed.

Chapter 2

To illustrate the clinical impact of port-site metastases, an extensive literature review of all reported port-site metastases until 1999 was done. In gynecological oncology, most port-site metastases seem to occur after diagnostic or therapeutic procedures for ovarian cancer. No reliable incidence of port-site metastases of gynecological cancers can be given. After laparoscopic surgery for colorectal cancer, 82 port-site metastases were described. They predominantly occurred in advanced disease (Duke's stages C and D) and the median interval until discovery is 6 months. An incidence of 0.9% is found when all series with more than 50 operated patients are analyzed. This corresponds with incidences of wound metastases known from open colorectal surgery (0.6 - 0.9 %). Gallbladder cancer is often an unexpected finding at pathological examination after cholecystectomy. In 3 large series of laparoscopic cholecystectomies, the incidences of gallbladder cancer range from 0.34 to 0.9 %. Port-site metastases were seen in 10 to 14%. In a series of 157 laparoscopic radical nephrectomies for renal cell carcinoma with a follow-up of 2.5 years, no port-site metastases were seen. Some case reports on port-site metastases after laparoscopic pelvic lymphadenectomy were found. Diagnostic laparoscopy for upper gastrointestinal cancers resulted in incidences of port-site metastases ranging from 0 to 7.5 %. They all developed in patients with disseminated disease or irresectable tumors at the time of laparoscopy.

Chapter 3

One of the theories on the etiology of port-site metastases is transportation of aerosolized tumor cells by carbon dioxide flow within the peritoneal cavity at laparoscopy. This was investigated by connecting the abdomens of two rats and establishing a pneumoperitoneum with a constant carbon dioxide flow from the first (the 'donor') to the second rat (the 'recipient'). A high number of tumor cells was injected intraperitoneally in the donors. This resulted in no or negligible tumor growth in the recipients and it was concluded that aerosolization of free intraperitoneal tumor cells is of limited importance in the pathogenesis of port-site metastases.

Chapter 4

Pressure of pneumoperitoneum may influence intraperitoneal tumor growth and thus is a potential factor in the etiology of port-site metastases. Intraperitoneal tumor growth was evaluated in rats following a 16 or a 4 mm Hg carbon dioxide pneumoperitoneum or abdominal wall elevation as control procedure. Peritoneal tumor growth in the 16 mm Hg pneumoperitoneum group was significantly increased. It was concluded that low insufflation pressures in oncologic laparoscopic surgery might be safer in terms of recurrence.

Chapter 5

Following the results of the experiment in chapter 4 and other experiments investigating pneumoperitoneum pressure, there is reason to suggest the existence of a critical pressure above which tumor growth increases instead of a linear correlation between and pressure and tumor growth. The pneumoperitoneum pressure experiment was repeated with an additional intermediate pressure group of 10 mm Hg and with helium and air instead of carbon dioxide. Tumor growth was similar in the 4 and 10 mm Hg groups and significantly higher in the 16 mm Hg group and there were no differences between the gases. These results support the idea of a critical pneumoperitoneum pressure between 10 and 16 mm Hg and therefore it seems advisable to keep the pressure under 12 mm Hg in oncological surgery.

Chapter 6

This chapter deals with two basic ideas on the occurrence of port-site metastases. One is that the degree of trauma at a port-site influences local tumor growth. The second is that gas and fluid leakage around a trocar causes transport of tumor cells to the leaking trocar wound. In the first experiment, small incisions were made in each abdominal quadrant and half of these wounds were crushed before introduction of 4 trocars in each rat. Tumor cells were injected intraperitoneally and a 20 minute pneumoperitoneum was administered. In the second experiment, 5 mm incisions were made in each abdominal half. In one incision, a 5 mm trocar and in the other, a 2 mm trocar was introduced. Tumor cells were injected intraperitoneally and during carbon dioxide pneumoperitoneum, there was leakage of 10 liters of gas along the 2 mm trocar. After 4 weeks, tumor growth at port-sites in the rats of both experiments was assessed. The crushed as well as the leaking sites showed significantly larger tumor deposits compared to the control sites.

Chapter 7

In this chapter, prevention of port-site metastases by cleaning of port-sites is evaluated. Several agents, known to have tumor inhibitory characteristics in other experiments, were used to clean port-sites in rats after injection of tumor cells and a period of carbon dioxide pneumoperitoneum. In two different experimental setups, no inhibitory or stimulating effect of cleaning with any agent was found.

Chapter 8

Ultrasonic surgery is claimed to cause less collateral trauma than diathermy, because the temperature at the tip of the instrument is much lower. The hypothesis that surgical trauma to the peritoneum stimulates intraperitoneal tumor growth was the basis for comparing an ultrasonic instrument with a diathermic instrument in an oncologic experiment in rats. Using the utrasonic or the diathermic instrument, standard peritoneal lesions were administered in rats before injecting a tumor cell suspension intraperitoneally. Tumor growth at the peritoneal lesions in the ultrasonically treated rats was significantly less than in diathermically treated rats.

CONCLUSIONS

- Aerosolization of free intra-abdominal tumor cells plays no role in the etiology of port-site metastases.
- A pneumoperitoneum with a pressure exceeding 10 mm Hg may increase adherence to peritoneal surfaces of tumor cells and / or stimulate tumor cell growth.
- Leakage of gas and fluid along a trocar during laparoscopic oncologic operations can induce port-site metastases.
- Cleaning of port-sites after a laparoscopic procedure does not appear to prevent port-site metastases.
- Less traumatic dissecting techniques, like ultrasonic instruments, can theoretically reduce local recurrence.

SAMENVATTING

Hoofdstuk 1

De geschiedenis en techniek van laparoscopische chirurgie worden beschreven. Vervolgens worden de ethische en praktische dilemma's rond de introductie van laparoscopische chirurgie in de oncologie behandeld. Tenslotte worden trocarwond metastasen en hun mogelijke oorzaken besproken.

Hoofdstuk 2

Als illustratie van het belang van trocarwond metastasen in de kliniek, werd een literatuur onderzoek gedaan naar alle beschreven trocarwond metastasen tot 1999. In de gynaecologische oncologie lijken de meeste trocarwond metastasen te ontstaan na diagnostische of therapeutische ingrepen voor ovarium kanker. Er is echter geen betrouwbare incidentie van trocarwond metastasen bij gynaecologische maligniteiten te berekenen. Na laparoscopisch chirurgische behandeling van colorectale kanker werden 82 trocarwond metastasen beschreven. Ze kwamen voornamelijk voor bij verder gevorderde stadia van kanker (Duke's stadium C en D) en de mediane tijd tot hun ontdekking is 6 maanden. Een incidentie van 0,9 % werd berekend na analyse van alle series met meer dan 50 geopereerde patiënten. Dit komt overeen met incidenties van wond metastasen na conventionele colorectale chirurgie (0,6–0,9 %). Galblaas carcinoom wordt vaak onverwachts gevonden bij pathologisch onderzoek na cholecystectomie. In drie grote series van laparoscopische cholecystectomieën varieert de incidentie van galblaaskanker van 0,34 tot 0,9 %. Trocarwond metastasen werden in 10 tot 14 % gezien. In een serie van 157 laparoscopische radicale nefrectomieën voor niercarcinoom met een follow-up van 2,5 jaar werden geen trocarwond metastasen gezien. Er werd wel wat casuïstiek gevonden over trocarwond metastasen na laparoscopische lymfklierdissectie van het bekken. Diagnostische laparoscopie wegens maligniteiten van de hogere gastrointestinale tractus resulteerde in incidenties van trocarwond metastasen van 0 tot 0,75 %. Deze ontwikkelden zich allemaal in patiënten bekend met gedissemineerde maligniteiten of inoperabele tumoren ten tijde van laparoscopie.

Hoofdstuk 3

Eén van de theorieën over de etiologie van trocarwond metastasen is het transport van zwevende tumorcellen via stroming van gas in de peritoneaal holte bij laparoscopie. Dit is onderzocht door de buiken van twee ratten te verbinden en een pneumoperitoneum aan te leggen met een constante stroom van koolstofdioxide gas van de eerste (de 'donor') naar de tweede rat (de 'ontvanger'). Een hoog aantal tumorcellen werd bij de donoren intraperitoneaal geïnjecteerd. Dit resulteerde in geen tot verwaarloosbare tumor groei in de ontvangers, zodat geconcludeerd kon worden dat aërosolisatie van vrije intraperitoneale tumorcellen een zeer beperkte rol heeft in de pathogenese van trocarwond metastasen.

Hoofdstuk 4

De druk van het pneumoperitoneum is een mogelijke factor in de etiologie van trocarwond metastasen. Na een koolstofdioxide pneumoperitoneum met een druk van 4 en 16 mm Hg en na een gasloze procedure met buikwand elevatie als controlegroep, werd de intraperitoneale tumorgroei in ratten geëvalueerd. De peritoneale tumorgroei na een 16 mm Hg pneumoperitoneum was significant verhoogd. Lage insufflatie drukken in de oncologische laparoscopische chirurgie geven mogelijk minder recidief.

Hoofdstuk 5

De resultaten van het in hoofdstuk 4 beschreven experiment en andere onderzoeken die betrekking hebben op de druk van het pneumoperitoneum, suggereren een kritische druk waarboven tumorgroei snel toeneemt in plaats van een lineaire correlatie tussen druk en tumor groei. Het experiment uit hoofdstuk 4 werd herhaald met een extra pneumoperitoneum druk groep van 10 mm Hg (dus met 4, 10, 16 mm Hg en een gasloze controle groep) en met helium en lucht in plaats van koolstofdioxide. Tumor groei was vergelijkbaar in de 4 en 10 mm Hg groep en significant hoger in de 16 mm Hg groep. Er waren geen verschillen tussen lucht en helium. Deze resultaten suggereren een kritische druk van het pneumoperitoneum tussen 10 en 16 mm Hg, waarboven de tumorgroei toeneemt. In oncologische laparoscopische operaties lijkt het verstandig om de pneumoperitoneum druk onder de 12 mm Hg te houden.

Hoofdstuk 6

Dit hoofstuk behandelt twee basale ideeën over het ontstaan van trocarwond metastasen. De eerste is dat de mate van schade bij een trocarwond de lokale tumorgroei beïnvloedt. De tweede is dat gas- en vloeistoflekkage rond een trocar tumorcellen naar de trocarwond toe transporteren. In het eerste experiment werden kleine incisies in alle kwadranten van de buik van ratten gemaakt, waarna de helft van deze wonden extra gekneusd werden vóór introductie van vier trocars in elke rat. Vervolgens werden tumorcellen intraperitoneaal geïnjecteerd en werd gedurende 20 minuten een pneumoperitoneum aangelegd. In het tweede experiment werden in beide helften van een rattebuik 5 mm incisies gemaakt. In één incisie werd een 5 mm trocar en in de andere werd een 2 mm trocar geïntroduceerd. Tumorcellen werden intraperitoneaal geïnjecteerd en gedurende het pneumoperitoneum was er een lekkage van 10 liter langs de 2 mm trocar. Na 4 weken werd in beide experimenten de tumorgroei bij de trocarwonden bepaald. Zowel de gekneusde als de lekkende trocarwonden hadden significant grotere tumoren dan de controle trocarwonden.

Hoofdstuk 7

In dit hoofdstuk wordt preventie van trocarwond metastasen door middel van reiniging van de wonden geëvalueerd. Enkele oplossingen met bekende tumor inhiberende eigenschappen werden gebruikt om trocarwonden in ratten te reinigen na intraperitoneale injectie van een tumorcel oplossing en een pneumoperitoneum gedurende een bepaalde periode. In twee verschillende experimenten werden geen inhiberende of stimulerende effecten op tumorgroei gevonden als gevolg van het reinigen met één van de oplossingen.

Hoofdstuk 8

Ultrasone chirurgie wordt geacht minder collaterale weefselschade te veroorzaken dan diathermie, omdat de temperatuur aan de punt van het instrument lager blijft. De hypothese dat chirurgisch trauma aan het peritoneum intraperitoneale tumorgroei stimuleert, vormde de basis om een ultrasoon instrument met een monopolair diathermisch instrument te vergelijken in een proefdierkundig oncologisch experiment. Met één van beide instrumenten werd gestandaardiseerde peritoneale wonden in ratten gemaakt, waarna

een tumorcelsuspensie intraperitoneaal geïnjecteerd werd. De tumorgroei bij de peritoneale wonden in de ultrasoon behandelde ratten was significant minder dan in de diathermisch behandelde ratten.

CONCLUSIES

- Aërosolisatie van vrije intraabdominale tumorcellen speelt geen rol in de etiologie van trocarwond metastasen.
- Een pneumoperitoneum met een druk hoger dan 10 mm Hg geeft mogelijk een extra stimulus aan adherentie van tumor cellen aan peritoneale oppervlakten en / of aan tumorcelgroei.
- Lekkage van gas en vloeistof langs een trocar tijdens een laparoscopische oncologische operatie kan trocarwond metastasen veroorzaken.
- Het reinigen van trocarwonden na laparoscopische operaties lijkt trocarwond metastasen niet te voorkomen.
- Het gebruik van minder traumatiserende technieken, zoals ultrasone instrumenten, kunnen in theorie lokaal recidief van de tumor verminderen.

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