

Effects of laparoscopy on intraperitoneal tumor growth and distant metastases in an animal model

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Background and aims. Laparoscopic surgery for colorectal cancer is currently being evaluated in humans. The aim of this study was to examine the effect of laparoscopy on intraperitoneal tumor growth and distant metastases in an animal model. We also examined the effect of combining laparotomy with laparoscopy and on infusing the peritoneal cavity with normal saline solution (NaCl), water, and sodium hypochlorite after laparoscopy on intraperitoneal tumor growth.

Material and methods. Female Fischer rats were given MtLn3 adenocarcinoma cells by intraperitoneal injection to produce intraperitoneal tumor growth and by tail vein injection to produce lung metastases. A pneumoperitoneum was then induced to a pressure of 8 mm Hg with carbon dioxide (CO₂), helium, or room air. After this, animals were allowed to either recover or underwent laparotomy or infusion of NaCl, water, or sodium hypochlorite before recovery, depending on the experiment. At 21 days all animals were killed and intraperitoneal tumor growth was assessed by counting the number of peritoneal and serosal nodules and by weighing the omental pad of tumor. Lung metastases were assessed by counting the number of metastases after fixation.

Results. Laparoscopy caused a marked intraperitoneal dissemination of tumor with a median of 17 (10 to 20) peritoneal and serosal nodules for CO₂, 19.5 (12.5 to 25) for helium, and 15.0 (9.5 to 17.7) for room air compared with 0 (0 to 1) for controls ($P < .0001$). The weight of omental tumor was also significantly increased ($P < .02$) in the CO₂, helium, and room air groups. Infusion with NaCl, water, or sodium hypochlorite had no effect on tumor dissemination after laparoscopy. The combination of laparoscopy and laparotomy caused a significant reduction ($P < .05$) in the number of peritoneal nodules but had no significant effect on omental tumor growth. Laparoscopy also had no effect on the number of pulmonary metastases induced compared with controls.

Conclusions. This study shows that laparoscopy promotes intraperitoneal dissemination of tumor. This effect is independent of the insufflating gas used and is not affected by use of a cytotoxic agent. The use of gasless laparoscopy should be encouraged by those undertaking curative laparoscopic surgery for colorectal cancer. (*Surgery* 1999;126:35-40.)

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IN 1991, AS LAPAROSCOPIC CHOLECYSTECTOMY was being accepted as a new technique in many centers, Jacobs et al¹ and Redwine and Sharpe² described laparoscopic right and left hemicolectomy, abdominoperitoneal resection, and stoma formation for both benign and malignant disease. This generated interest in laparoscopic colorectal surgery elsewhere, and reports from both the United Kingdom and the United States³⁻⁶ confirmed the feasibility of these

techniques. Although the ability to perform laparoscopic colorectal resection cannot be questioned, controversy regarding its oncologic safety remains. Enthusiasts claim that the principles of oncologic surgery are strictly adhered to, with resection margins, tumor clearance, and lymph node harvest comparable to those of conventional open surgery.⁷ However, as yet no long-term follow-up of patients after laparoscopically assisted surgery for colorectal cancer is available.

Of particular concern has been the high rate of wound recurrence and port-site metastases reported after a short follow-up period.⁸ The first reports of port-site metastases were observed after staging procedures for intraperitoneal cancers.⁹⁻¹³ Such lesions have also been reported after cholecystectomy¹⁴⁻¹⁷ and now there are many reports of port-site metastases after colorectal cancer surgery.^{12-14,16-24}

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In a recent review of the literature by Wexner and Cohen²⁵ the authors estimated that 6.5% of patients undergoing curative colorectal cancer surgery have port-site metastases. Although this is likely to be an overestimate, it is clear that wound recurrence after open surgery remains rare and is thought to be less than 1%.²⁶

Little is known about the effect of laparoscopy on intraperitoneal tumor growth. The aim of this study was to examine the effect of laparoscopy with different gases on tumor growth after intraperitoneal and intravenous injection of tumor in an animal model. We also examined the effect of combining laparoscopy and laparotomy on tumor growth in this model. Finally, because normal saline solution (NaCl) and occasionally sterile water are used as lavage fluids during laparoscopic surgery, we examined the effect of infusion of these fluids and the cytotoxic agent sodium hypochlorite on intraperitoneal tumor growth after laparoscopy.

MATERIAL AND METHODS

Animals. Female Fischer rats aged 8 to 12 weeks were obtained from Harlan UK Ltd. Rats were maintained in the University of Glasgow animal facility under appropriate conditions and were allowed food and water ad libitum. All work was performed under the provisions of the "Animals (Scientific Procedures) Act 1986" and the supervision of the Home Office.

Tumor cells. The cell line was the MtLn3 clone of the rat adenocarcinoma cell line originally derived by Neri and Nicholson, M. D. Anderson Hospital and Tumour Institute, Houston, Tex.²⁷ This is derived from the mammary adenocarcinoma line 13762NF, induced by dietary administration of 7,12-dimethylbenz[a]anthracene, and is known to have a high metastatic potential. It is syngenic for the F344 Fischer rat. Cells have been kept in liquid nitrogen. Each batch of frozen cells was passaged no more than 6 times to prevent phenotypic drift. The medium used was Hanks F10 and Dulbecco's modified Eagles' medium (Life Technologies, Paisley) with 10% fetal calf serum and L-glutamine. Cells were grown to confluence in 5% carbon dioxide (CO₂) at 37°C. They were then washed with phosphate-buffered saline solution and trypsinized with 0.2% trypsin-EDTA (ethylenediaminetetraacetic acid) solution (Life Technologies). They were washed 3 times in medium by centrifugation at 1200 revolutions/min and resuspended in fresh medium at the appropriate concentration. Viability was assessed by trypan blue exclusion. All cell suspensions were greater than 90% viable and were used within 2 hours of preparation.

Study 1: effect of laparoscopy on intraperitoneal tumor spread. The pattern of intraperitoneal spread of MtLn3 has been previously described.²⁸ Briefly, after intraperitoneal injection a tumor nodule develops at the injection site. In addition, there are multiple small parietal peritoneal deposits, particularly in the subdiaphragmatic area. The omentum becomes diffusely infiltrated with tumor, but there are few or no visceral deposits and no solid-organ metastases.

Seventy animals were divided into 4 groups. General anesthetic was induced with oxygen-halothane and animals were maintained with 2% halothane by a nose cone. All animals received an intraperitoneal injection of 1×10^4 cells of MtLn3 in 1 mL of medium. Group 1 (controls) had intraperitoneal tumor injection and anesthetic only. Group 2 had a CO₂ pneumoperitoneum induced by inserting an 18-gauge needle in the midline subumbilically and insufflating to a pressure of 8 mm Hg (Wolff automatic insufflator). This pressure produces a tense pneumoperitoneum without causing diaphragmatic splinting in the nonintubated animal. Group 3 had a helium pneumoperitoneum induced in the same manner, whereas group 4 had a pneumoperitoneum induced with room air. A pressure of 8 mm Hg was maintained for 15 minutes. After this time the abdomen was allowed to deflate through the 18-gauge needle, which was then removed and the animals recovered. Any remaining low-pressure pneumoperitoneum was reabsorbed over the succeeding 24 hours. All animals were killed at 21 days, or sooner if the clinical condition deteriorated.

Assessment of intraperitoneal tumor spread. Tumor spread was assessed by careful counting of peritoneal and serosal nodules and weighing the excised omentum. Specimens were sent for histopathologic study to confirm the presence of tumor.

Study 2: effect of laparoscopy and laparotomy on intraperitoneal tumor growth. Because most laparoscopic procedures for colorectal cancer combine laparoscopy with a small laparotomy wound, we examined the effects of both laparoscopy and laparotomy on intraperitoneal tumor growth. Thirty animals were randomized into 3 groups. All were anesthetized as above and received 1×10^4 MtLn3 cells in 1 mL of medium. Group 1 received a CO₂ pneumoperitoneum as previously described to a pressure of 8 mm Hg. Group 2 underwent laparotomy only. Group 3 had a pneumoperitoneum with CO₂ for 15 minutes, followed by a laparotomy. At the end of the procedure the wound was closed in layers with plain catgut suture to the

Table I. Effect of laparoscopy on intraperitoneal tumor growth

Group	No.	Omental tumor (g)	Peritoneal nodules
Control	20	4.0 (3.2-5.9)	0 (0-1.0)
CO ₂	20	7.5 (5.8-8.8)	17.0 (10.0-20.0)
Helium	20	6.1 (5.0-8.3)	19.5 (12.5-25.7)
Room air	10	7.4 (3.8-8.6)	15.0 (9.5-17.7)
Statistical significance*		<i>P</i> = .018	<i>P</i> = .0001

*Compared with controls.

peritoneum and polyglactin mesh (Vicryl, Ethicon, Somerville, NJ) to the skin; all animals recovered. All animals were killed at 21 days, or sooner if the clinical condition indicated. The extent of tumor growth was assessed as previously described, and an assessment of wound tumor was made.

Study 3: effect of laparoscopy followed by peritoneal infusion on intraperitoneal tumor growth. Forty F344 rats were randomized into 4 groups. All were anesthetized as above and received 1×10^4 MtLn3 tumor cells in 1 mL as an intraperitoneal injection. Group 1 (controls) underwent a CO₂ pneumoperitoneum as previously described. Group 2 underwent pneumoperitoneum followed by infusion of 2 mL of warm NaCl into the peritoneal cavity. Group 3 underwent the same procedure with sterile water, whereas group 4 rats were infused with a solution of 0.3% sodium hypochlorite (Milton). Sodium hypochlorite was chosen for this experiment because it had previously been shown to be the most effective cytotoxic solution both in vitro and in vivo against the MtLn3 cell line.²⁸ Infusions were left within the abdominal cavity. At the end of the procedure all animals were recovered. Animals were killed at 21 days or sooner if clinical conditions indicated, and tumor load was assessed as above.

Study 4: effect of laparoscopy on lung metastases. In this model, after a tail vein injection of MtLn3 tumor cells, lung metastases will develop in all animals. Forty-five animals were randomized into 3 groups: control, CO₂ pneumoperitoneum, and helium pneumoperitoneum. All had general anesthesia induced with oxygen-halothane in a Perspex box and were maintained with 2% halothane by a nose cone. All received a tail vein injection of 1×10^4 cells in 0.2 mL of medium. Pneumoperitoneum was then induced as in the first experiment. All animals were killed at 21 days, or sooner if the clinical condition indicated, and lung metastases were counted.

Assessment of lung metastases. The metastases were counted in the manner of Wexler.²⁹ Briefly, the excised lungs were washed with water and inflated with India ink through the trachea. They were

then fixed in alcoholic Bouin's solution. Metastases became white and could be counted at 24 hours.

Statistics. Data were collated with use of the Statistics Package for Social Sciences (SPSS, Chicago, Ill). All values are expressed as medians with interquartile ranges. Peritoneal nodules, lung metastases, and omental weights were compared with the Mann-Whitney *U*-Wilcoxon rank-sum test.

RESULTS

Study 1: effect of laparoscopy on intraperitoneal tumor spread. All animals survived to 21 days and all had diffuse intraperitoneal tumor involving the parietal peritoneal surfaces with gross infiltration of the omentum and with blood-stained ascites at autopsy. Both the CO₂ helium and room air laparoscopy groups had significantly greater omental and peritoneal involvement compared with controls (Table I). There was no significant difference between the CO₂ helium and room air groups. Histopathologic study confirmed the presence of adenocarcinoma at all sites tested.

Study 2: effect of laparoscopy and laparotomy on intraperitoneal tumor growth. All animals survived to 21 days and had blood-stained ascites with disseminated intraperitoneal tumor as before. In addition, all animals in the laparotomy group had diffuse infiltration of the wound with tumor. There was no significant difference in the amount of omental tumor in any group nor in the degree of tumor infiltration in the wound. However, both the laparotomy alone group and the laparotomy plus laparoscopy group had significantly fewer peritoneal deposits compared with the laparoscopy alone group (Table II).

Study 3: effect of laparoscopy followed by peritoneal infusion on intraperitoneal tumor growth. All animals survived to 21 days and had blood-stained ascites and tumor growth within the peritoneum as described. There were no significant differences in the amount of tumor growth between control and infusion groups (Table III).

Study 4: effect of laparoscopy on lung metastases. All animals survived to 21 days and at autopsy all groups had evidence of lung metastases with-

Table II. Effect of laparoscopy and laparotomy on intraperitoneal tumor growth

Group	No.	Omental tumor (g)	Peritoneal nodules
Laparoscopy	10	7.2 (4.6-8.1)	13.0 (6.5-18.5)
Laparotomy	10	8.1 (5.7-11.8)	3.0 (1.0-4.0)
Combined	10	5.9 (4.6-8.0)	5.0 (2.5-6.5)
Statistical significance*		NS	$P = .05$

NS, Not significant.

*Compared with laparoscopy group.

Table III. Effect of laparoscopy followed by peritoneal infusion on intraperitoneal tumor growth

Group	No.	Omental tumor (g)	Peritoneal nodules
Laparoscopy	10	4.6 (3.5-6.1)	14.5 (10.5-17.0)
Water	10	5.3 (3.4-6.8)	11.0 (6.7-14)
NaCl	10	6.1 (3.2-6.2)	10.5 (6.0-13.5)
Sodium hypochlorite	10	6.9 (6.6-7.9)	12.0 (10.5-13.2)
Statistical significance*		NS	NS

NS, Not significant.

*Compared with laparoscopy group.

Table IV. Effect of laparoscopy on lung metastases

Group	No.	Lung metastases
Control	15	5 (3-12)
CO ₂	15	9 (7-10)
Helium	15	6 (6-14)
Statistical significance*		NS

NS, Not significant.

*Compared with control group.

out gross dissemination of disease. There were no significant differences in the number of lung metastases observed among the CO₂, helium, and control groups (Table IV).

DISCUSSION

Our results show that laparoscopy causes dissemination of tumor cells and promotes tumor growth within the peritoneal cavity in an animal model. This effect was independent of the gas used and was significantly reduced when laparoscopy was followed by laparotomy. It was not affected by infusion of sterile water, NaCl, or the cytotoxic agent sodium hypochlorite.

Interestingly, laparoscopy could not be demonstrated to have any effect on distant metastases, indicating that local factors may be the predominant mechanism whereby laparoscopy promotes intraperitoneal dissemination of tumor. It is likely that there is aerosolization of tumor cells within the enclosed space of the peritoneal cavity. In the clinical setting, with repeated introduction and withdrawal of instruments allowing gas to leak through ports, cells may be drawn to the port sites. The

pressure of insufflating gas may force these cells into the peritoneum and port-site wound, allowing them to seed and grow. The fact that in this model laparoscopy insufflation followed by laparotomy significantly reduces the amount of peritoneal tumor growth with little effect on omental tumor growth suggests that prolonged pressure is required to allow seeding and growth of peritoneal nodules to take place.

The concept of tumor cell aerosolization is supported by Knolmayer et al³⁰ and their large animal model, which showed that there is a constant aerosol of epithelial cells within the peritoneum during laparoscopy and that the number of cells escaping increases with increasing intra-abdominal pressure. Champault et al³¹ have shown the presence of clumps of cells in gas exhausted from laparoscopy, the so-called "chimney effect," although no malignant cells have yet been identified in their studies.

Several other workers have investigated the effect of a pneumoperitoneum on intraperitoneal tumor growth with use of cell suspension and solid tumor in small animal models. Jacobi et al³² showed that the simple act of creating a CO₂ pneumoperitoneum caused increased tumor growth within the peritoneal cavity. Work by Bouvy et al^{33,34} using both cell suspension and solid tumor models achieved similar results. In contrast, the results of Hubens and Eyskens³⁵ showed no difference in the rate intraperitoneal tumor growth between anesthetic controls and CO₂ insufflation. They scored peritoneal involvement with use of a scale described by Eggermont et al³⁶ that assessed

intraperitoneal involvement on the basis of visual inspection and does not differentiate between tumor growth within the omentum and involvement of the parietal peritoneum. It is interesting to note that, in common with our results, they did not find any significant differences in tumor growth between laparoscopy and laparotomy.

Bouvy et al³³ also investigated the effects of gasless laparoscopy on intraperitoneal tumor growth with use of a solid tumor model and showed that both laparotomy and CO₂ pneumoperitoneum produced significantly greater intraperitoneal tumor growth than gasless laparoscopy did. Port-site metastases were also significantly reduced in the gasless group compared with the CO₂ pneumoperitoneum group. Similar results were achieved by the same authors with a cell suspension of CC531.³⁴ These results are supported by Watson et al,³⁷ who demonstrated that the incidence of port-site metastases in a rat model was reduced after gasless laparoscopy compared with insufflation with CO₂.

In comparing the effects of laparotomy, laparoscopy, and a combined laparoscopy-laparotomy procedure, our results showed that laparoscopy alone produced significantly more parietal peritoneal tumor deposits than did either laparotomy or the combined procedure. There was no significant difference in omental tumor weight among the 3 groups nor in the amount of wound involvement between the laparotomy and combined groups. It is well known that laparotomy has a permissive effect on tumor growth.³⁸ However, studies generally demonstrate that this effect is less with laparoscopy.^{32-35,39} These studies have examined the effects of laparoscopy on tumor growth at a site remote from the peritoneal cavity, such as the animal's flank, and may help to explain some of the differences between their findings and our study.

It might be expected that infusion of the abdominal cavity with sterile water or sodium hypochlorite would result in a reduction of intraperitoneal tumor growth. We have previously shown that sodium hypochlorite is cytotoxic *in vitro* and *in vivo* against the MtLn3 cell line.²⁸ Further work is therefore required to identify a cytotoxic agent that will prevent the growth of tumor that occurs with laparoscopic insufflation. In the meantime, avoidance of insufflation by the use of gasless laparoscopy should be practiced by surgeons undertaking laparoscopic resection for colorectal cancer.

In conclusion, we have shown that laparoscopy promotes the dissemination of tumor growth within the peritoneal cavity and that this effect is independent of the gas used. Use of a cytotoxic agent or

lavage with water or saline solution has no effect on tumor cell dissemination by laparoscopy. Further work is required to investigate the role of instrument and cannula contamination on port-site metastases and to examine what effect laparoscopy may have on peritoneal and serosal surfaces that promote tumor growth.

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