Study on incisional implantation of tumor cells by carbon dioxide pneumoperitoneum in gastric cancer of a murine model

WANG Hao, ZHENG Min-Hua, ZHANG Hao-Bo, ZHU Jian, HE Jian-Rong, LU Ai-Guo, JI Yu-Bao, ZHANG Min-Jun, JIANG Yu, YU Bao-Ming and LI Hong-Wei

Subject headings stomach neoplasms; colonic neoplasms; cell movement; carbon dioxide pneumoperitoneum; murine model

INTRODUCTION
Port-site recurrence after laparoscopic tumor surgery is a frequent complication in cancer operations, such as gallbladder, stomach, ovary and colon[1-5]. The incidence of port-site recurrence after laparoscopic colectomy ranged from 1.1% to 6.3%, in contrast to a 0.68% tumor wound recurrence rate in patients undergoing curative open colectomy[6-9]. The possible mechanisms proposed were: ① contaminated laparoscopic instruments passing in and out of the port frequently; ② increased exfoliated cancer cells from laparoscopic manipulation; ③ adhered tumor cells by pneumoperitoneum[9-12]. Some experiment reported that desufflation related to seeding of port wounds via a stable suspension of tumor cells in CO2 gas was an unlikely cause of port tumors, some reported that desufflation related to seeding of port wounds via a stable suspension of tumor cells in CO2 gas was an unlikely cause of port tumors, some supported a direct intraperitoneal seeding of exfoliated tumor cells as its etiology and the instruments passing in and out of the port may play an important role in local recurrence[13-15]. The colon tumor cells were more common since laparoscopic colectomy was wildly performed.

The purpose of this study was to determine whether CO2 pneumoperitoneum could increase tumor implants in the port site.

MATERIALS AND METHODS

Materials
A 5mm laparoscopic port (5 mm trocar) was inserted in the left iliac fossa and Veress needle was placed in the right iliac fossa, below which was the injection site of malignant cells. Then the right iliac fossa port was used for insufflation, and another was used for desufflation, through the same collection device. Laparoflator was made in Germany (laparoflator electronic 3509 WEST GmbH).

Colon cancer cell line LoVo and gastric cancer cell line SGC-7901 (from Shanghai Institute of Digestive Surgery) were suspended in liquid culture media and divided into 2 groups: ① the liquid tumor cell suspension contained 1 million cells in 1mL volume (10^6 cells/L); ② the liquid tumor cell suspension contained 10 thousand cells in 1mL volume (10^4 cells/L). The concentration of cells in the suspension was determined to be greater than 95 percent by trypan blue exclusion. Continuous flow of CO2 was allowed by leaving the outflow port opened during insufflation, intraperitoneal pressure was maintained at the desired level via constant insufflation during co ntinuous flow studies.

Methods
Male Sprague-Dawley rats (250 g-350 g, from Shanghai Experimental Animal Center) were anesthetized with 25 g/L sodium barbitone (1 µL/g). Abdomens were shaved and prepared with bromo-geramine. Animals then received a right lower quadrant intraperitoneal injection of 1 mL of a suspension of SGC-7901 gastric cancer cells or LoVo colon cancer cells (10^6/L, 10^4/L), respectively. Veress needle and 5mm trocar were placed in the abdomen and served as port sites. There were 4 pairs of groups for LoVo or SGC-7901 (4 rats for each group): ① continuous pneumo of...
2 kPa (5 min) at gas flow of 5 L/min for 5 min with \((10^7/L, 10^9/L)\) cells injected; \(\textcircled{2}\) continuous flow (5 L/min) of CO2 with \((10^7/L, 10^9/L)\) cells injected, maintaining a pressure of 4 kPa for 5 min inside the peritoneal cavity; \(\textcircled{3}\) continuous flow (5 L/min) of CO2 with \((10^7/L, 10^9/L)\) cells injected, maintaining a pressure of 2 kPa inside the peritoneal cavity for 60 min; and \(\textcircled{4}\) continuous flow (5 L/min) of CO2 with \((10^7/L, 10^9/L)\) cells injected, maintaining a pressure of 4 kPa for 60 min inside the peritoneal cavity. At the end of the experiments, a peritoneal washing sample was cultured as a cell viability control. All collection dishes were incubated at 37 °C and 50 mL/L CO2 concentration for one week, then detected under microscopy to demonstrate whether tumor cells existed or not.

**RESULTS**

Continuous CO2 pneumoperitoneum with different number of cell injection in LoVo & SGC-7901 cell line were shown in Table 1 and Table 2, respectively. After one week of incubation, in the group of 5 L/min, continuous CO2 flow of 4 kPa for 60 min with \(10^7/L\) SGC-7901 cell injected, it demonstrated tumor growth in 3 of 4 dishes when compared with the same experimental condition in LoVo cell. All 4 peritoneal washing samples also showed tumor growth, whereas other dishes showed none.

| Cell number | No. of rats | Pressure (kPa) | Duration (min) | Tumor growth |
|-------------|-------------|----------------|----------------|--------------|
| \(10^7/L\)  | 4           | 2              | 5              | 0/4          |
| \(10^9/L\)  | 4           | 2              | 5              | 0/4          |
| \(10^7/L\)  | 4           | 4              | 5              | 0/4          |
| \(10^9/L\)  | 4           | 4              | 5              | 0/4          |
| \(10^7/L\)  | 4           | 4              | 60             | 0/4          |
| \(10^9/L\)  | 4           | 2              | 60             | 0/4          |
| \(10^7/L\)  | 4           | 4              | 60             | 0/4          |
| \(10^9/L\)  | 4           | 2              | 60             | 0/4          |
| Control     | 2           |                |                | 2/2          |

**Table 2 Results in continuous flow pneumo with SGC7901 cell injection**

| Cell number | No. of rats | Pressure (kPa) | Duration (min) | Tumor growth |
|-------------|-------------|----------------|----------------|--------------|
| \(10^7/L\)  | 4           | 2              | 5              | 0/4          |
| \(10^9/L\)  | 4           | 2              | 5              | 0/4          |
| \(10^7/L\)  | 4           | 4              | 5              | 0/4          |
| \(10^9/L\)  | 4           | 4              | 5              | 0/4          |
| \(10^7/L\)  | 4           | 2              | 60             | 0/4          |
| \(10^9/L\)  | 4           | 2              | 60             | 0/4          |
| \(10^7/L\)  | 4           | 4              | 60             | 0/4          |
| \(10^9/L\)  | 4           | 4              | 60             | 0/4          |
| Control     | 2           |                |                | 2/2          |

**DISCUSSION**

Laparoscopic surgery has been carried out nationwide in patients with cancer of the gastrointestinal tract despite relatively high incidence of port site recurrence after curative resection\(^{[17]}\). Several clinical reports have proposed that recurrence may be caused by direct implantation of the tumor cells, whereas the proof is still uncertain. Many experimental studies of colon carried out more than those in gastric cancer\(^{[16]}\).

Our design was to evaluate and compare the incidence of port site recurrence by direct seeding of either colon or gastric cancer cells. We injected LoVo cells into the mice and found none of the 32 mice had tumor growth in the dishes, but when injected SGC-7901 cells into the mice with \(10^9/L\) SGC901 cells and pneumoperitoneum pressure 4 kPa for 60 min, 3 out of 4 dishes showed tumor cells growth. The gastric cancer cell line SGC-7901 was more likely to cause port-site recurrence than colon cancer LoVo cell line. This may partly be due to the difference of tumor metastatic behavior. It had been reported that the capacity of gastric cancer cell implantation in the peritoneum was much easier than that of the colon cancer cells\(^{[18,19]}\). Our finding corroborated the above conclusion. The pneumoperitoneum pressure in the abdominal cavity and its duration played an important role in the development of port-site recurrence of gastric cancer cells.

The mechanism for tumor cell port-site implantation may be explained as follow: \(\textcircled{1}\) tumor cell exfoliation by surgical manipulation of the tumor; \(\textcircled{2}\) contaminated laparoscopic instruments frequently passing in and out of the ports; \(\textcircled{3}\) tumor cell viability, number of cells, duration, pneumoperitoneum pressure and the metastatic nature of tumor cells; \(\textcircled{4}\) surgery induced immunosuppression facilitating tumor growth at the port-site wounds\(^{[13,20]}\). Thus significant effort should be strived for to prevent tumor growth in the port wound. It has been suggested that all instruments should be routinely wiped on withdrawal from a port with a cytotoxic agent (povidone-iodine) and a similar agent flushing the laparoscopic port before withdrawal. The external aspect of the port should be sprayed and wound liberally irrigated with a cytotoxic agent\(^{[17]}\).

**REFERENCES**

1. Alexander RJ, Jaques BC, Mitchell KG. Laparoscopically assisted colectomy and wound recurrence. Lancet, 1993;341:249-250
2. Drouard F, Delamarre J, Capron JP. Cutaneous seeding of gallbladder cancer after laparoscopic cholecystectomy. N Engl J Med, 1991;325:1316
3 Cava A, Roman J, Gonzalez QA, Quintela A, Martin F, Aramburo P. Subcutaneous metastasis following laparoscopy in gastric adenocarcinoma. *Eur J Surg Oncol*, 1990;16:63-67

4 Clair DG, Lautz DB, Brooks DC. Rapid development of umbilical metastases after laparoscopic cholecystectomy for unsuspected gallbladder carcinoma. *Surgery*, 1993;113:355-358

5 Gleeson NC, Nicosia SV, Mark JE, Hoffman MS, Cavanagh D. Abdominal wall metastases from ovarian cancer after laparoscopy. *Am J Obstet Gynecol*, 1993;169:522-523

6 Ramos JM, Gupta S, Anthone GJ, Ortega AE, Simons AJ, Beart RW Jr. Laparoscopy and colon cancer. Is the port site at risk. A preliminary report. *Arch Surg*, 1994;129:897-900

7 Vukasin P, Ortega AE, Greene FL, Steele GD, Simons AJ, Anthone GJ, Weston LA, Beart RW Jr. Wound recurrence following laparoscopic colon cancer resection. *Dis Colon Rectum*, 1996;39:S20-S23

8 Hughes ES, McDermott FT, Polglase AL, Johnson WR. Tumor recurrence in the abdominal wall scar tissue after large bowel cancer surgery. *Dis Colon Rectum*, 1983;26:571-572

9 Nduka CC, Monson JR, Menzies Gow N, Darzi A. Abdominal wall metastases following laparoscopy. *Br J Surg*, 1994;81:648-652

10 Fusco MA, Paluzzi MW. Abdominal wall recurrence after laparoscopic assisted colectomy for adenocarcinoma of the colon: report of a case. *Dis Colon Rectum*, 1993;36:858-861

11 Ciocco WC, Schwartzman A, Golub RW. Abdominal wall recurrence after laparoscopic colectomy for colon cancer. *Surgery*, 1994;116:842-846

12 Umpleby HC, Fernor B, Symes MD, Williamson RC. Viability of exfoliated colorectal carcinoma cells. *Br J Surg*, 1982;71:659-663

13 Iwanaka T, Arya G, Ziegler MM. Mechanism and prevention of port site tumor recurrence after laparoscopy in a murine model. *J Pediatr Surg*, 1998;33:457-461

14 Whelan RL, Sellers GJ, Allendorf JD, Laird D, Bessler MD, Nowygrod R. Treat MR. Trocar site recurrence is unlikely to result from aerosolization of tumor cells. *Dis Colon Rectum*, 1996;39:S7-S15

15 Allardyce R, Morreau P, Bagshaw P. Tumor cell distribution following laparoscopic colectomy in a porcine model. *Dis Colon Rectum*, 1996;39:S47-S52

16 Hubens G, Pauwels M, Hubens A, Vermeulen P, Van-Marcck E, Eyskens E. The influence of a pneumoperitoneum on the peritoneal implantation of free intraperitoneal colon cancer cells. *Surg Endosc.*, 1996;10:809-812

17 Hewett PJ, Thomas WM, King G, Eaton M. Intraperitoneal cell movement during abdominal carbon dioxide insufflation and laparoscopy. *Dis Colon Rectum*, 1996;39:S7-S15

18 Asao T, Nagamachi Y, Morinaga N, Shitara Y, Takenoshita S, Yazawa S. Fucosyltransferase of the peritoneum contributed to the adhesion of cancer cells to the mesothelium. *Cancer*, 1995;75(6 Suppl):1539-1544

19 Asao T, Yazawa S, Kudo S, Takenoshita S, Nagamachi Y. A novel ex vivo method for assaying adhesion of cancer cells to the peritoneum. *Cancer Lett.*, 1994;78:57-62

20 Murthy SM, Goldschmidt RA, Rao LN, Ammirati M, Buchmann T, Scanlon EF. The influence of surgical trauma on experimental metastasis. *Cancer*, 1989;64:2035-2044