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

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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[6-8]. 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 sreported that desufflation related to seeding of port wounds via a stable suspension of tumor cells in CO_2 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 CO_2 pneumoperitoneum could increase tumor implants in the port site.

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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 injecti on 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 (10^9 cells/L); ② the liquid tumor cell suspension co ntained 10 thousand cells in 1mL volume (10^7 cells/L). The concentrati on of cells was calculated with a hemocytometer (Fischer Scientific, Pittsburg, PA) and then appropriately diluted to achieve the final concentration. Liquid culture media were RPMI 1640 containing 10 percent fetal bovine serum. Cell viability control culture and cell viability of each tumor cell preparation were determined to be greater than 95 percent by trypan blue exclusion. Continuous flow of CO_2 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 A minal 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 (10^7/L, 10^9/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; ② continuous flow (5 L/min) of CO^2 with (10^7/L, 10^9/L) cells injected, maintaining a pressure of 4 kPa for 5 min inside the peritoneal cavity; ③ continuous flow (5 L/min) of CO_2 with (10^7/L, 10^9/L) cells injected, maintaining a pressure of 2 kPa inside the peritoneal cavity for 60 min; and ④ continuous flow (5 L/min) of CO_2 with (10^7/L, 10^9/L) cells injected, maintaining a pressure of 4kPa for 60min 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 ℃ and 50 mL/L CO_2 concentration for one week, then detected under microscopy to demonstrate whether tumor cells existed or not.

RESULTS

Continuous CO_2 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 CO_2 flow of 4 kPa for 60min with 10^9/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.

Table 1 Results in continuous flow pneumo with LoVo cell injection

Cell number	No. of rats	Pressure (kPa)	Duration (min)	Tumor growth
10^9/L	4	2	5	0/4
10^7/L	4	2	5	0/4
10^9/L	4	4	5	0/4
10^7/L	4	4	5	0/4
10^9/L	4	2	60	0/4
10^7/L	4	2	60	0/4
10^9/L	4	4	60	0/4
10^7/L	4	4	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
10^9/L	4	2	5	0/4
10^7/L	4	2	5	0/4
10^9/L	4	4	5	0/4
10^7/L	4	4	5	0/4
10^9/L	4	2	60	0/4
10^7/L	4	2	60	0/4
10^9/L	4	4	60	3/4
10^7/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[1,7]. 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: ① tumor cell exfoliation by surgical manipulation of the tumor; ② contaminated laparoscopic instruments frequently passing in and out of the ports; ③ tumor cell viability, number of cells, duration, pneumoperitoneum pressure and the metastatic nature of tumor cells; ④ surgery induced immuno-suppression 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 withdrawl. The external aspect of the port should be sprayed and wound liberally irrigated with a cytotoxic agent[17].

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