Rapid flow carbon dioxide laparoscopy disperses cancer cells into the peritoneal cavity but not the port sites in a new rat model

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Abstract

Background: The role of carbon dioxide (CO2) in the pathogenesis of tumor recurrence after laparoscopy remains controversial. Using a new rat model, we studied the effect of different CO2 flow rates on the dispersal of free cancer cells.

Methods: A novel model of desufflation without trocar was developed, and 55 Fischer rats were randomized into three flow groups: group A (rapid, 0.67 l/min; n = 20), group B (slow, 0.44 l/min; n = 20), and group C (gasless, n = 15). We vented CO2 via a portless surgical valve that filtered cells. After the abdominal wall had been suspended to create space, half of the animals in each group (nonrecovery) received 7.5 x 10^6 immunolabeled rat colon cancer cells (RCC2) intraperitoneally, whereas the other half (recovery) received 7.5 x 10^6 viable RCC2 before insufflation or gasless laparoscopy. Nonrecovery animals were killed after 20 l of insufflation. Parietal peritoneal and port-site specimens were examined for RCC2 by fluorescence microscopy (FM) and flow cytometry (FC). The recovery animals were killed at 4 weeks for evidence of wound recurrence.

Results: Nine of 10 nonrecovery animals in A had RCC2 on FM or FC, as compared with 2 animals in each of the nonrecovery groups B and C (p = 0.018, Fisher’s exact test). Two of the nine animals in group A also had RCC2 in their portless valves. Two recovery (A) animals developed wound recurrence as compared with none in the other groups (p = 0.315).

Conclusion: In this model, rapid CO2 flow dispersed free cancer cells into the peritoneal cavity, but not into the port sites, thus supporting a role for CO2 in the intra-peritoneal dispersal of free cancer cells, but not in wound recurrence.

Key words: Aerosolization — Animal model — Laparoscopy — Port-site recurrence — CO2 pneumoperitoneum — Cancer

The potential for iatrogenic tumor dissemination during laparoscopy, particularly at the port site or in patients with early disease, causes concern. The etiology of these recurrences is thought to be multifactorial, but experimental simulations have yielded conflicting evidence. Many have noted enhanced tumor growth and metastasis with carbon dioxide (CO2) pneumoperitoneum, as compared with laparotomy or gasless controls [3, 7, 13, 18, 20]. In a murine model of laparoscopic splenectomy, Lee et al. [16] showed that port-site recurrences (PSR) decreased with increased experience at using the procedure, which could explain the early cases reported during the learning curve.

Despite collective experience in laparoscopic surgery and the restriction of curative laparoscopic cancer resections to trial protocols, unusual recurrences continue to be reported [6, 17, 22–24, 26]. There is evidence to suggest that the use of trocars is associated with increased PSR rates [10, 12], and the addition of CO2 pneumoperitoneum has been shown in some studies to triple the rates of PSR [14] and to cause greater cellular dispersal [15, 21]. This was corroborated clinically by Cavina et al. [4], who found that labeled autologous red blood cells deposited in the bed of the gallbladder before laparoscopic cholecystectomy diffused widely in CO2, but not in a gasless group of patients.

Although air-borne cellular dispersal (aerosolization) has been demonstrated [5, 11, 15], its contribution to the dissemination of cancer and PSR is unclear. There is a pressure-dependent egress of cells during CO2 laparoscopy [15], but few data are available on the relationship between the rate of gas flow and the risk of cellular dispersal. Clinically, gas flow varies throughout
laparoscopic surgery, and large volumes of CO₂ are used during more complex and prolonged procedures, such as laparoscopic colectomy. Rapid flow occurs when pneu-

moperitoneum is reduced abruptly, and this may be associated with the egress of cells in droplets and aerosol. On the other hand, cells may be entrapped during slow gas leakage around ports (The so-called “chimney effect”). Because CO₂ currently is the insufflant of choice, its role in the dispersal of cancer cells needs further clarification.

To determine whether different flow rates aerosolized free cancer cells, and whether this contributed to wound recurrence, we developed a new rat model of laparoscopic desufflation without the use of cannulae.

Materials and methods

Cancer cell lines an immunolabeling

Transplantable rat colon cancer cells (RCC2) derived from a parent line established by Borman et al. [2] were maintained continuously in standard aseptic culture (RPMI1640 with 10% fetal calf serum and glutamine) at 37°C and 5% CO₂. Cells were counted using a modified Neub avoided hemocytometer (Gallenkemp, Leicestershire, UK), and their viability was tested by trypan blue exclusion (TBE). A concentration of 7.5 x 10⁶ cells suspended in 0.5 ml of phosphate-buffered saline (PBS) maintained on ice was used for the procedures. Immunolabeling was performed at room temperature using monoclonal antibodies to the RCC2 (JB39.4) previously raised by one of the authors (C.-Y. Y.) [1].

As a negative control, an indifferent (anti-fast myosin) antibody also was tested. Fluorescent secondary labeling was performed before injection by incubating the cells at room temperature with fluorescein isothiocyanate (FITC) conjugated fluorocytosine (FC) specific goat antihuman IgG and rat serum protein (Sigma Immuno Chemicals, St Louis, MO, USA). Cells were viewed with a fluorescence microscope at an excitation wavelength of 490nm.

Portless Valve Model

To eliminate the contribution of ports and instruments to wound contamination and subsequent port-site recurrence, we developed and tested a flap valve mechanism in the abdominal wall that vented gas while entrapping any floating cells. A 4-mm transverse skin incision was made in the epigastrum and undermined 1 cm proximally and 1 cm distally, creating two pockets. A 4 to 5-mm midline incision was made through the muscle into the peritoneal cavity in each pocket so that the deep incisions were completely concealed by the overlying skin flap. This configuration entrapped cells from effluent gas with each insufflation–desufflation cycle, without impeding gas flow (Fig. 1).

Pilot experiments

Two separate test animals, each were injected with 1 million RCC2 in the lateral abdominal wall and 1 million in the peritoneal cavity. The animals were observed over 4 weeks to confirm allogpatibility. To determine the optimum temperature for immunolabeling, the process was performed separately at 25°C and at 37°C with primary antibody (P25, P37) and control antibody (C25, C37). A mixture of peritoneal washout and blood cells was used as a negative control for the flow cytometry (FC) while a mixture of peritoneal washes, labeled and unlabeled RCC2, served as a positive control. The ability of the portless valve to vent large volumes of gas, as described later, also was tested at both slow and rapid flow rates.

Laparoscopic procedures

For the laparoscopic procedures, 55 highly inbred, pathogen-free, 6-week-old weanling male Fisher 344 rats (200–250g) were used according to an approved protocol (PPL 70/4133) and the care guidelines of the UK Home Office. The animals were assigned randomly to one of three flow groups: rapid (group A, n = 20), slow (group B, n = 20), or zero flow (group C, n = 15). With the animals under general anesthesia, the portless valve was constructed in the epigastrum. A 5-mm incision in the suprapubic area was made for laparoscopy and insufflation. The anterior abdominal wall then was suspended with several sutures tied to the rails of an operating table to create working space and separate the peritoneal from the visceral peritoneum. A 5-mm disposable Endopath trocar (Ethicon, Edinburgh, UK) was inserted and secured to the insufflation port. A 4-mm 0° arthroscope connected to a light source was used to view the abdomen, and great care was taken throughout the procedure not to allow any contact between the suspended abdominal wall or the port and the seeded visceral peritoneum.

Under direct vision 7.5 x 10⁶ RCC2 cells (viable or immunola-

beled) were carefully injected through a marked spot on the pos-
terolateral abdominal wall to seed only the visceral peritoneum. The animals then were assigned randomly to one of three groups. In the two insufflation groups, CO₂ was administered using an electronic high-flow insufflator (Smith & Nephew, Cambridge, UK) until 20 l were used. A positive result was defined as the presence of labeled RCC2 on FC or fluorescence microscopy (FM) or both for nonre-
covery group, or histologic evidence of tumor in the recovery co-

Fig. 1. Schematic section of the portless valve model used. A single port at the caudal end delivers CO₂ from the visceral peritoneum (vp) to the parietal peritoneum (pp). Cells also are trapped in the muscle vents (V1 and V2) and under the skin flap before the gas escapes through the skin incision (E). The flaps F1 and F2 serve as “filters” for floating cells in the effluent gas. Note that retrograde leak (b) also occurs at the inlet port. (s, skin; m, muscle wall)

Group A: rapid flow (n = 20).

A high-flow rate of 0.67 l/min at 4 to 6 mmHg vented 20 l of CO₂ through the valve over 30 min. Animals receiving labeled cells (non-
recovery, n = 10) were killed under anesthesia by intracardiac injec-
tion of pentobarbitone. The suspended portion of the abdominal wall 1 cm clear of the injection site was carefully excised and its peritoneal surface washed several times with PBS. The material was centrifuged and resuspended in 5 ml of PBS. Half of this was examined by FC, and the other half was cytospun and examined by FM. The portless valve and the insufflation port were also excised and imprinted on poly-l-
lysine–coated glass slides for FM. The samples were then processed by standard methods of paraffin blocks, stained with hematoxylin and eosin, and viewed with light microscopy and FM. Animals receiving viable cells (recovery, n = 10) had their wounds sutured and observed for 4 weeks before they were killed. All the animals and tissue samples were examined by a pathologist (J. S. C.-B.) who was unaware of the procedures performed.