Peritoneal Irrigation with Povidone-Iodine Solution After Laparoscopic-Assisted Splenectomy Significantly Decreases Port-Tumor Recurrence in a Murine Model

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PURPOSE: The development of port-wound tumor recurrences has raised questions regarding the safety of laparoscopic methods for the resection of malignancies. The cause and the incidence of abdominal-wall tumor recurrences remain unknown. It is also not clear how to avoid or lower the incidence of port-tumor recurrences. The purpose of the current study was to determine the impact of abdominal irrigation with povidone-iodine on the port-wound tumor incidence in a murine model. METHODS: A splenic tumor model was used for this study. To establish splenic tumors, female BALB/c mice (N = 48) were given subcapsular splenic injections of a 0.1 ml suspension containing 10^5 026 colon adenocarcinoma cells via a left-flank incision at the initial procedure. Seven days later, the animals with isolated splenic tumors (100 percent) were randomly assigned to one of three groups: 1) control, 2) saline irrigation (saline), or 3) povidone-iodine irrigation. All animals underwent laparoscopic mobilization of the spleen using a three-port technique, intra-abdominal crushing of the tumor, followed by extracorporeal splenectomy via a subcostal incision. No irrigation was performed for control group animals. In the saline irrigation group, the subcostal incision was closed and pneumoperitoneum was re-established. The abdominal cavity was irrigated with 5 ml of normal saline for 60 seconds before instrument removal. In the povidone-iodine irrigation group, similar abdominal irrigation was performed, using 0.25 percent povidone-iodine. Attempts were made to recover completely the irrigation for both irrigation groups. Seven days after the splenectomy, animals were killed and inspected for abdominal-wall tumor implants. RESULTS: There were significantly more animals with at least one port-tumor recurrence in the control group than in the povidone-iodine group (P = 0.007). Although not statistically significant, the number of animals with port-wound tumors was higher in the saline group than in the povidone-iodine group (P < 0.08). There was no significant difference between the saline group and the control group. When each port site was considered independently, the incidence of port-wound tumors (number of ports with tumors per total number of ports) was significantly lower in the povidone-iodine group than in both the control (P = 0.00001) and saline groups (P = 0.03). The incidence of port-wound tumors was also significantly lower in the saline group compared with the control group incidence (P = 0.03). CONCLUSIONS: Abdominal irrigation with dilute povidone-iodine solution significantly reduced the number of animals with port-tumor recurrences. Abdominal irrigation with saline was also effective in reducing the incidence of port-wound tumor formation when each port was considered separately. However, povidone-iodine irrigation was much more effective than saline irrigation in preventing port-wound tumor formation. [Key words: Povidone-iodine; Peritoneal irrigation; Port-site tumor recurrence; Port-wound tumors; Laparoscopy; Splenic tumor model]


The cause of port-site tumors is presently unknown. It is also not clear what can be done at the time of surgery to avoid port-tumor recurrence. To investigate these issues we have recently developed a murine solid-tumor model in which an isolated tumor is established via a splenic injection at the initial procedure. The resulting primary tumors were then resected under a variety of conditions, using a number of different techniques. Using this model, it is possible to assess how tumor cells are shed in addition to determining what happens to the liberated cells. In the initial study using this model, we demonstrated that traumatic handling of the splenic tumor at the time of open splenectomy resulted in significantly more port-wound tumors than after open splenectomy performed using careful, meticulous technique. In contrast, the addition of CO2 pneumoperitoneum after splenectomy done with either traumatic or meticulous technique had no impact on the rate of port-wound tumor formation. In the second study,
using the same splenic tumor model, we compared the incidence of port-tumor recurrence after laparoscopic-assisted (LAS) vs. open splenectomy (OS). This experiment was performed in two separate trials. We found that port-tumor recurrence rate was significantly higher in the LAS than in the OS group in the first trial. In the second trial, with increased experience, the incidence of port-tumor recurrences in the LAS group fell to that of the OS group. The results of these two studies suggest that the spillage or liberation of cells from the primary tumor is the critical event in wound-tumor formation. They also suggest that the best way to prevent post-wound tumors is carefully to avoid handling or manipulating the tumor during resection and thus avoid cell spillage. The use of tumoricidal agents before tumor cells have the opportunity to implant into the wounds may also be an effective strategy in further reducing port-tumor recurrence. The purpose of this study was to determine the impact of peritoneal irrigation with dilute povidone-iodine solution in reducing implantation potential of tumor cells.

MATERIALS AND METHODS

The study protocol was submitted to and approved by the Columbia University Institutional Animal Care and Use Committee.

Tumor Cell Preparation

The murine colon 26 adenocarcinoma (C-26) cell line was utilized for this study. The C-26 tumor line is syngeneic to the BALB/c mouse strain, and the tumor is grown as a monolayer in plastic tissue culture flasks (Fisher Scientific) in RPMI 1640 medium supplemented with 2 mM L-glutamine, 10 percent fetal calf serum, 150 U/ml penicillin, and 150 mg/ml streptomycin. Before the experiment, the cells were washed once, trypsinized (2.5 percent), and then resuspended in the above-mentioned culture media at a final concentration of 1 × 10^6 cells per milliliter. Viability of the cells was verified by use of the trypan blue exclusion test before and after injection of the cell suspension into the study animals. Using this method, viability always exceeded 95 percent.

Pilot Study to Determine Optimal Concentration for Abdominal Irrigation

A total of 10^6 C-26 colon adenocarcinoma cells in 200 μl of media were incubated for 30 minutes with one of the following concentrations of povidone-iodine: 1) 1 percent, 2) 0.2 percent, 3) 0.1 percent, 4) 0.075 percent, 5) 0.05 percent, or 6) 0 percent. Viability of tumor cells was determine by use of a tetrazolium salt (MTS)/phenazine methosulfate nonradioactive, colorimetric, cell-proliferation assay (Promega, WI). In this assay viable tumor cells convert MTS into a formazan that is soluble in tissue-culture media. The absorbance of formazan at 490 nm can be read using an enzyme-linked immunosorbent assay reader. The conversion of MTS to a soluble tetrazolium salt is accomplished by a dehydrogenase enzyme found in metabolically active cells. The quantity of formazan as measured by absorbance of 490 nm is directly proportional to the number of viable cells in the culture.

Splenectomy Study

A total of 48 female BALB/c mice underwent splenic injection. The mice were anesthetized with intraperitoneal ketamine (50 mg/kg) and xylazine (5 mg/kg) and their abdomens were shaved and prepared. A left-flank incision was made, through which the spleen of each animal was externalized and then injected with 10^5 C-26 colon adenocarcinoma cells in 0.1 ml of media. This optimal concentration of cells was determined via a pilot study performed at the time of the first splenic tumor study. After the needle was removed, the spleen was swabbed with 70 percent alcohol to kill any extravasated tumor cells. The spleen was then replaced in the abdomen and the flank incision was stapled closed. Seven days later, all animals underwent laparoscopic mobilization of the spleen using a three-port technique. Insufflation was accomplished via a 20-gauge angiocatheter placed percutaneously in the subxiphoid area. A 3 mm laparoscopic camera was inserted via a right lower quadrant incision and secured. Carbon dioxide gas was insufflated to a pressure of 4 to 6 mmHg. Next, two 14-gauge laparoscopic ports were inserted in the right upper and left lower quadrants, respectively, under direct visualization via two small incisions. A 16-gauge grasping forceps was inserted via the right upper quadrant port and a pair of straight scissors was introduced through the left lower quadrant port. Using these instruments, the adhesions and attachments of the spleen were carefully dissected and the spleen mobilized under direct vision, preserving only the short gastric and hilar vessels. A pair of laparoscopic straight scissors was then used to incise across the longest and the shortest diameters of the exposed tumor surfaces (simulating spillage of tumor cells). The animals were assigned to study groups randomly.