Port-Site Recurrence Reproduced in the VX-2 Rabbit Carcinoma Model: An In Vivo Model Comparing Laparoscopic Port Sites and Open Incisions

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ABSTRACT

Background: The use of advanced laparoscopy remains controversial in the field of surgical oncology because the potential for port-site recurrence may violate sound oncologic principles. Two mechanisms are theorized to be the cause of port-site recurrences: first, indirect contamination caused by pneumoperitoneum, aerosolization, or intraperitoneal spread, and second, direct contamination by physical trocar seeding.

Methods: A VX-2 carcinoma cell suspension was transferred under the left renal capsule of 31 rabbits with either an open flank incision (16) or laparoscopy (15). Animals were observed for tumor recurrence at the video port, the working port, and the open incision. Intraoperative findings and necropsy were used to document recurrence.

Results: The open incision technique resulted in local tumor recurrence in 1/16 animals with 16/16 viable intraabdominal tumors. The laparoscopic technique resulted in 0/15 video port-site recurrences and 9/15 working port-site recurrences, with 14/15 viable intraabdominal tumors. Recurrence at the laparoscopic working port occurred more frequently than in the open ($P < 0.02$) or laparoscopic video port groups ($P < 0.007$). No significant difference existed in recurrence between the open incision and the laparoscopic video port ($P > 0.5$).

Conclusions: Laparoscopic port-site recurrences can be reproduced using the transplantable VX-2 rabbit carcinoma model. In the VX-2 model, trocar recurrence is the result of direct contamination via surgical instrumentation of viable tumor cells. The effect of the pneumoperitoneum or intraperitoneal cytological spillage (indirect contamination) does not have any effect on trocar recurrence. This model can be used to test and improve laparoscopic techniques to minimize the risk of port-site recurrence. Until technological advances have eliminated the risk of trocar recurrences, direct contact between malignant cells and laparoscopic instruments should be performed with caution.

Key Words: Port-site recurrence, Port-site metastases, Port-site implantation, Tumor seeding, Pneumoperitoneum, CO_2, Laparoscopic surgery, VX-2 tumor.

INTRODUCTION

Laparoscopic surgery has proven benefits for the surgical treatment of numerous benign diseases. Advantages of laparoscopic surgery include decreased hospital stay, shortened recovery time, reduction in postoperative pain, and a more rapid return to regular activities. In some animal studies, the traumatic effects of surgery have been found to be immunosuppressive. As a corollary, minimally invasive techniques may preserve important immunological reserve of the patient harboring cancer.[1] Laparoscopic surgery has been used primarily for staging and palliation of abdominal or thoracic tumors.[2-5] To date, the use of advanced laparoscopy for treatment/resection of cancer is not widely accepted.[6-8] Numerous questions exist regarding the extent of resection, the effects of pneumoperitoneum, and finally port-site recurrence. Laparoscopic port-site recurrences have been reported in both benign[9] and malignant diseases.[10,11] Unfortunately, despite numerous animal studies and case reports, the etiology of port-site recurrence is still not well understood. Port-site recurrences are theorized to occur via indirect contamination (pneumoperitoneum, aerosolization, and intraperitoneal spread) or direct contamination (physical trocar seeding).[12] Understanding the mechanism of port-site recurrence is imperative if laparoscopy is to be used in cancer treatment.

MATERIALS AND METHODS

Our goal was to establish a minimally invasive method of achieving VX-2 tumor transfer into the subcapsular renal

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space in rabbits. We made every attempt to achieve zero operative mortality, minimal postoperative weight loss, and no extrarenal tumor growth. The Institutional Animal Care and Use Committee of Eisenhower Army Medical Center reviewed and approved the protocol.

VX-2 Rabbit Carcinoma

First described in 1940,[13] the VX-2 rabbit carcinoma is a reproducible transplantable malignancy. The VX-2 model readily produces tumors that penetrate surrounding connective tissues at the primary inoculation site. Invaded tissues exhibit an inflammatory response or desmoplastic reaction. The tumor forms a white-tan nodule or mass with centralized necrosis. Histologically, the tumor has been described as an anaplastic squamous cell carcinoma, revealing cells in a sheet-like pattern separated by a thin stroma consisting of capillaries and inflammatory cells. The tumor readily metastasizes to lung and lymph nodes, but metastases to other organs are uncommon.[14]

Tumor Preparation

To establish carriers for the VX-2 tumor, male New Zealand White rabbits weighing 3.5 to 4.5 kg were lightly anesthetized with ketamine (30 mg/kg) and xylazine (2 mg/kg) intramuscularly. After shaving and sterilely preparing the right lateral hindleg, 0.5 mL of a VX-2 tumor suspension previously stored at -150C was injected into the right hindleg. Approximately 2 weeks later, the tumor had grown to 2.0 to 2.5 cm in size. After euthanasia, viable tumor fragments were harvested in a sterile fashion from the hindleg musculature and placed in a petri dish containing Supplemented Dulbecco's Modified Eagle Medium (Modified DMEM) on ice. Supplemented DMEM contains DMEM without phenol red, sodium pyruvate, or sodium bicarbonate (Gibco BRL), 1X MEM nonessential amino acids (Sigma, St. Louis, MO, USA), 10% heat inactivated fetal bovine serum (FBS) (Equitech-Bio, Inc., Ingram, TX, USA), 1 x 10^{-8}M 1, 24-dihydroxy vitamin-D3 (CalBiochem, LaJolla, CA, USA), 100 U penicillin and 0.1 mg streptomycin per mL (Gibco BRL), 50 ug/mL gentamicin (Gibco BRL), 1.25 ug/mL fungizone (Gibco BRL), 0.6% sodium bicarbonate (Sigma), 15mM HEPES (Sigma), 0.11 mg/mL sodium pyruvate (Sigma), and 10^{-5}M ß-Mercaptoethanol (Sigma).

The tumor was dissected in a sterile fashion into 1 to 2 mm^3 fragments. These fragments were placed into a sterile 50-mL tube containing 20 mL Supplemented DMEM, and allowed to stand on ice for 30 minutes. A tumor cell suspension was prepared by gently passing the tissue fragments through a 40-mesh cell dissociation grinder kit (Sigma). The suspension was centrifuged at 700 RPM for 3 minutes, after which the supernatant was discarded. The pellet was resuspended with 0.9% PBS and subjected to 2 additional washings. Trypan blue solution 0.5% was added to an aliquot of cell suspension, and a cell count by hemocytometer was performed. The resultant cell solution was prepared to an approximate viable cell density of 1.14 x 10^6 cells/mL and placed on ice. This suspension was then thawed prior to its use.

Operative Technique

Thirty-one male New Zealand White rabbits were anesthetized intramuscularly with ketamine (35 mg/kg), xylazine (4 mg/kg), and glycopyrrolate (0.01 mg/kg IM); anesthesia was maintained via isoflurane (1-3% in 100% oxygen) via orotracheal intubation. The abdomen of each rabbit was shaved and prepared for surgery in a sterile fashion. Animals were randomized to either an open (n = 16) or laparoscopic (n = 15) approach group as described below. All incisions were closed in layers. Absorbable suture (3-0 Vicryl, Ethicon, Cincinnati, OH) was used to close the fasciomuscular layer in interrupted figure of eight fashion. Subcuticular skin closure was then performed using 4-0 Vicryl (Ethicon, Cincinnati, OH).

Open Approach

Rabbits in the open group received a 4-cm left flank incision. Sharp dissection was performed until the left kidney was exposed. The kidney was then elevated into the wound and a 14-gauge intravenous catheter was used to inject 1.5 to 2.0 mL of tumor suspension beneath the renal capsule. All attempts were made to avoid gross tumor spillage into the peritoneal cavity or flank incision by placement of laparotomy sponges around the exposed kidney. No irrigation was used, and the incision was closed in layers as previously described.

Laparoscopic Approach

Rabbits in the laparoscopic group underwent placement of a 5-mm trocar at the lower midline using standard Hassan technique. Carbon dioxide (CO_2) pneumoperitoneum was established and maintained at 8 to 10 mm Hg. A pediatric cystoscope was inserted through the 5-

mm trocar and used for a video port (VP). Manual external manipulation along with blunt and sharp dissection facilitated the laparoscopic exposure of the left kidney. Once the lower pole was identified, a second disposable 3-mm port was placed under visual guidance to serve as a working port (WP). A 14-gauge needle was inserted via the WP and advanced under the left renal capsule. Once appropriately positioned, 1.5 to 2.0 mL of the tumor cell suspension was injected through this needle. Within the limitations of laparoscopy, all attempts were made to reproduce the open technique. The WP was removed under direct visualization. Gentle external pressure ensured that the pneumoperitoneum was not released from the WP site. The VP was used to release the pneumoperitoneum, after which the VP was removed. No effort was made to limit the release of gas or fluid from either port after the VP was removed. All attempts were made to avoid gross tumor spillage into the peritoneal cavity or either port incision. No irrigation was used, and the incision was closed in layers as previously described.

Postoperative Care

Immediately after surgery, all animals were administered buprenorphine (0.02 mg/kg) intramuscularly to control postoperative pain. Following tumor implantation, a second exploration was performed to confirm successful tumor transplantation between postinjection day 7 and 14. Detailed intraoperative evaluation was performed to document renal and extra-renal tumor growth. Extrarenal sites of most importance were open flank and trocar incisions. Time of first incisional or port-site recurrence was documented in each case. Animals were euthanized if no renal tumor was present, tumor burden was excessive, or 6 weeks had elapsed following confirmation of successful tumor transplant. The final postmortem examination was performed to confirm that no local recurrence was missed.

Statistical Method

Differences between the various groups: WP, VP, and open incision were evaluated using Fisher's exact test. Results were considered statistically significant at $P < 0.05$.

RESULTS

Thirty-one rabbits underwent VX-2 tumor transfer with no operative deaths. Successful VX-2 tumor transfer beneath the kidney was confirmed in 30 animals. The

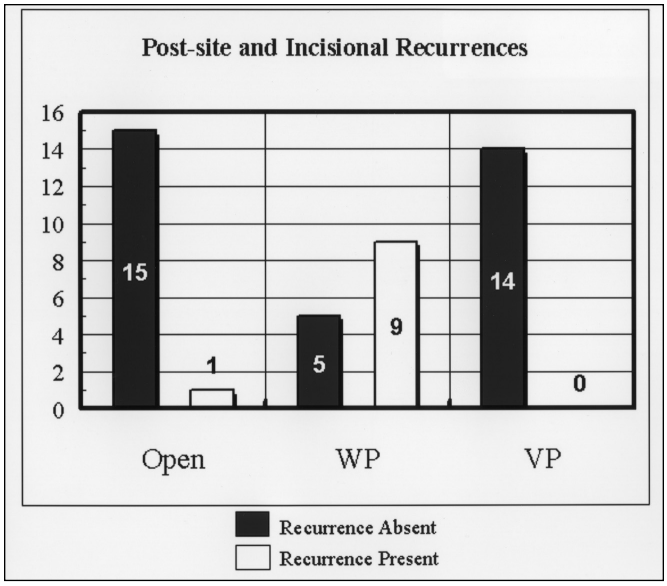

Figure 1. Recurrence rate by the various techniques used: open and laparoscopic. Open technique had incisional recurrence rate of 6%. In the laparoscopic groups the working port (WP) had a 64% recurrence rate, but the video port (VP) had a 0% recurrence rate.

open incision technique resulted in a renal tumor in 16 of 16 injections. The open technique resulted in a local incisional recurrence in 1/16 animals. The laparoscopic technique gave rise to 14 renal tumors out of 15 injections. Only 1 animal failed to produce a renal tumor. The 1 animal that failed to produce a viable renal tumor did not have either video or working port-site recurrence. Failure to produce a baseline renal tumor may have been due to improper handling of the VX-2 media; thus, only animals with viable renal tumors were used in statistical analysis. As such, the laparoscopic technique resulted in 0/14 video port-site recurrences and 9/14 working port-site recurrences **(Figure 1)**. Recurrence at the laparoscopic working port occurred more frequently than in the open group ($P < 0.02$) or laparoscopic video port ($P < 0.007$). No significant difference existed in recurrence between open incision and laparoscopic video port ($P > 0.5$).

All animals were confirmed to have recurrence at incision or port sites by intraoperative exploration or necropsy, or both **(Figure 2)**. Computed tomography (CT) scanning was initially used to document recurrence but was found to be unreliable; many animals without CT evidence of local disease were found on later exploration

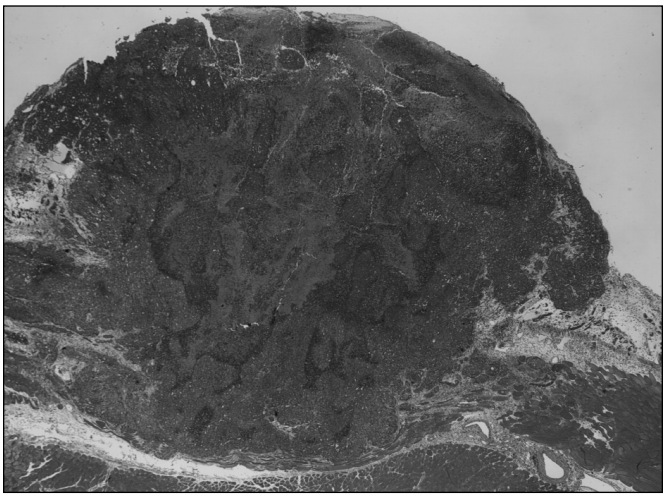

Figure 2. Hematoxylin and eosin (H&E) section demonstrating trocar recurrence in the abdominal wall. Note tumor is small and contained within the muscular fibers. All tumors presented within 20 days of contamination.

Table 1.
Observation Time Between Different Groups.

	Interval(mean)	Range
Open (all)	30.9 days	7-49
Laparoscopic (all)	16.3	7-33
Wound Recurrence(lap)	12.8	7-20
No Wound Recurrence(lap)	21.6	9-33
No Wound Recurrence(open)	31.1	7-49

Table 2.
Comparison of Colorectal and Pancreatic Cancers.

	Positive peritoneal cytology	% with stage IV disease
Colon	3% to 30%	70% to 100%
Pancreas	12% to 22%	100%

to have local disease. The mean interval to confirmed positive recurrence was 12.8 days with a standard error of 1.8. The mean interval to confirmed negative recurrence was 29.1 days with a standard error of 3.6. Animals in all groups that failed to demonstrate recurrence were observed for a longer period of time to ensure that a subclinical recurrence was not missed. No recurrences occurred after 20 days **(Table 1)**.

DISCUSSION

Surgical oncologists have resisted incorporating laparoscopy into the management and treatment of the cancer patient. Presently, this tool remains underutilized except in selected indications: staging and palliation. Numerous concerns remain: whether to include the extent of resection, effects of CO_2 pneumoperitoneum, and finally trocar recurrences. Data will be forthcoming in the near future regarding safety and efficacy of laparoscopic resection of colorectal cancers.[15] Other malignancies will await further trials well into the future.

The pathogenesis of trocar recurrences remains an open question. Indirect contamination of the trocar site may occur when free intraperitoneal cancer cells are pushed from the abdominal cavity to the trocar site via a pressure gradient. This is also referred to as the "chimney" effect. To study this phenomenon, laparoscopists have used intraperitoneal injection of tumor cells in various animal models.[16] These models assume that cancer cells are free floating in the peritoneal cavity, and thus reproduce carcinomatosis or stage IV disease. Interestingly, most patients with intraabdominal cancer have negative cytology at time of diagnosis and treatment. For example, in colorectal and pancreatic cancer only 1 in 3 patients has positive cytology; furthermore, those with positive cytology tend to have more advanced disease **(Table 2)**.[17-20] The intraperitoneal injection models have implications for advanced disease states, but may not accurately model laparoscopy in cancer patients who present with stage I, II, or III disease. Furthermore, this theory fails to explain how trocar recurrences have occurred in thoracoscopic and gasless laparoscopy.[13]

Direct contamination of the trocar site may occur when surgical instruments convey cancer cells from the abdominal cavity to the port-site.[16] This theory requires that viable cancer cells undergo iatrogenic transfer and deposition into the subcutaneous tissue at the port site. The cancer cells may arise from direct contact with a solid tumor or may be picked up in surgically contaminated

Table 3.
Literature Search.

Author	Journal	Year	Technique
Bouvy	*Ann Surg*	96	Solid mass intraperitoneal
	Br J Surg	97	Subcapsular renal injection
Watson	*Arch Surg*	97	SQ tumor laceration
Lee	*Surg Endosc*	98	Subcapsular splenic injection
	Dis Colon Rectum	98	Subcapsular splenic injection

peritoneal fluid. To best reproduce the direct contamination model, cytology negative, solid tumor models are required. Solid tumor models are harder to reproduce and as such animal models are few in number **(Table 3)**.[21-25] Most of these models require that tumor implantation be followed by surgical manipulation. Multiple surgical procedures, tumor variability, and surgeon experience can affect trocar recurrence. Simplified solid organ models are still needed to study the trocar recurrence phenomena.

The VX-2 rabbit model can produce a solid organ malignancy and results in reproducible incisional and trocar recurrences. This model demonstrated an open incisional recurrence rate of 6%. This is far greater than the historical incisional recurrence rate found in human colorectal cancers, which falls between 0.6% and 0.8%.[26,27] This ten fold increase in open recurrence rate attests to the aggressive nature of the VX-2 tumor. More important than the open incisional recurrence rate is the laparoscopic trocar recurrence rate. In the colorectal literature, the incidence of laparoscopic trocar recurrences has been reported to be between 0% and 21%, but recent series place the incidence between 0% and 2%.[13,15,16] In the VX-2 model, the trocar recurrence rate was 64% at the WP. This high recurrence rate at the WP makes this model ideal for testing and perfecting surgical techniques. By reproducing the direct contamination model at 1 trocar, efforts can be focused on how to limit subcutaneous implantation. New trocar removal techniques and local treatments (surgical or chemical) can be tested using this model. A second key finding was the 0% VP recurrence rate. If trocar recurrences were the result of indirect contamination from the intraperitoneal VX-2 cells, then the VP and WP would be equally affected. Of note, the pneumoperitoneum was released primarily via the VP. When the animals underwent formal exploration, carcinomatosis was not seen. The VX-2 model did not produce intraperitioneal contamination to any clinically significant degree. The CO_2 pneumoperitoneum did not have any independent effect on trocar recurrence. This implies that diagnostic laparoscopy is safe and should result in minimal trocar recurrence risk in the cancer patient without positive cytology or stage IV disease. Direct tumor contact was the deciding factor between recurrence and recurrence-free ports. The ability to reproduce a trocar recurrence without contaminating the peritoneal cavity makes the model more applicable to human cancers.

CONCLUSION

VX-2 trocar recurrence is the result of direct contamination between the surgical instruments and viable tumor cells. VX-2 animal model reproduces trocar recurrence in 64% of working ports but 0% of video ports. The effect of indirect contamination, pneumoperitoneum, or intraperitoneal cytological spillage did not have any effect on recurrence rate. This negative cytology, solid tumor model can be used to improve laparoscopic techniques and minimize trocar recurrences. At present, malignant cell contact with laparoscopic instruments should be performed with caution.

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