ORIGINAL ARTICLE

Efficacy of Cytotoxic Agents for the Prevention of Laparoscopic Port-Site Metastases

Susan J. Neuhaus, MBBS; David I. Watson, MBBS, FRACS; Tanya Ellis, BSc; Thomas Dodd, MBBS; Allan M. Rofe, PhD; Glyn G. Jamieson, MS, FRCS, FRACS

Background: Recent experimental studies support initial clinical impressions that laparoscopic surgery for malignant neoplasms may be associated with an increased incidence of metastases to port sites. This study investigated in an experimental model the influence of cytotoxic agents (administered intraperitoneally or intramuscularly) on the development of port-site metastases following laparoscopic surgery.

Methods: Seven days after the implantation of an adenocarcinoma in the left abdominal flank, 72 Dark Agouti rats underwent laparoscopy with carbon dioxide insufflation, instillation of an intraperitoneal agent, and intraperitoneal tumor laceration within the following study groups (12 rats in each group): (1) control (no intraperitoneal instillation); (2) intraperitoneal instillation of isotonic sodium chloride solution (0.9%); (3) intraperitoneal instillation of povodine-iodine (1:10 dilution of povidine-iodine and isotonic sodium chloride solution); (4) intraperitoneal instillation of methotrexate (0.125 mg of methotrexate in 3 mL of isotonic sodium chloride solution); and (5) intraperitoneal instillation of aqueous chlorhexidine acetate. Twelve additional rats underwent laparoscopic tumor laceration following intramuscular injection of 0.125 mg of methotrexate (no intraperitoneal agent). Rats were killed 7 days after the procedure, and the wounds were examined histologically by a blinded histopathologist for the presence of tumor metastases.

Results: No tumor was found in any port site following the intraperitoneal administration of povidine-iodine ($P = .04$). In contrast, port-site metastases developed in the control group (5 [41.7%] of 12), the isotonic sodium chloride solution group (4 [33.3%] of 12), the chlorhexdine group (4 [33.3%] of 12), the intraperitoneal methotrexate group (2 [16.7%] of 12), and the parenteral methotrexate group (5 [41.7%] of 12).

Conclusions: The results of this study suggest that the development of metastases to port sites following laparoscopic surgery may be prevented by the intraperitoneal instillation of diluted povodine-iodine. Other agents failed to influence the incidence of port-site metastases. Further studies are needed to determine if these findings can be applied to humans.

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From the The Royal Adelaide Centre for Endoscopic Surgery, Royal Adelaide Hospital (Drs Neuhaus, Watson, and Jamieson and Ms Ellis), and the Institute of Medical and Veterinary Science (Drs Dodd and Rofe), Adelaide, Australia.

THE INCREASING number of case reports of port-site metastasis, as well as the recently reported outcomes of several experimental studies, suggest that the laparoscopic manipulation of malignant neoplasms is associated with an increased incidence of metastasis to port sites.[1,2] Irrespective of whether wound contamination with tumor cells occurs due to either direct contamination from laparoscopic instruments that have been in contact with malignant tissue or indirect contamination from tumor cells aerosolized in the insufflation of gas,[3-5] it is possible that the application of topical intraperitoneal cytotoxic agents or the use of parenteral agents as adjuvant therapy may prevent metastasis by killing tumor cells that have been liberated by laparoscopic manipulation.[1,6-8] The use of these agents would then overcome one of the principal impediments to laparoscopic cancer surgery.[1,2,5]

Studies[5,9] in our department using a tumor model in an immunocompetent syngeneic rat strain, as well as studies[3,10,11] from other workers, have investigated the mechanism of tumor metastasis to port sites. The results have confirmed that port-site metastases are a problem and that possible preventive strategies, such as gasless laparoscopy, can be tested using this approach.[5] To investigate the possibility that either intraperitoneal or parenteral cytotoxic agents might decrease the incidence of port-site metastases following laparoscopic surgery, these strategies were tested in an established experimental model.

MATERIALS AND METHODS

We injected the musculature of the left abdominal flank of 72 male *Dasyprocta* rats with an adenocarcinoma cell suspension. Previous work[5] using this model has confirmed that the tumor grows reliably to form a mass approximately 20 to 25 mm in maximal diameter after 7 days and that this mass extends into the abdominal cavity while still covered by peritoneum. The tumor is readily visible during laparoscopy.[5]

Seven days after tumor implantation the rats underwent a laparoscopic procedure with carbon dioxide insufflation, tumor laceration, and instillation of an intraperitoneal agent in 1 of the following 6 groups, with 12 rats in each group: (1) control group (no intraperitoneal instillation); (2) intraperitoneal instillation of isotonic sodium chloride solution; (3) intraperitoneal instillation of povodine-iodine; (4) intraperitoneal instillation of methotrexate; (5) intraperitoneal instillation of chlorhexidine acetate; and 1 additional group (12 rats) underwent laparoscopic tumor laceration following preoperative intramuscular injection of methotrexate (no instillation of intraperitoneal agent).

Earlier work[12] using the tumor cell line confirmed the in vivo sensitivity of the tumor cells to methotrexate.

The tumor cell line (DAMA) used for these studies originally was derived from a spontaneously occurring mammary adenocarcinoma native to the Dark Agouti rat strain.[13] The method of cell supsension has been described in detail previously.[5] Each rat was injected with 0.15 mL of tumor suspension (2×10^7 viable cells in 200 µL of sterile phosphate-buffered isotonic sodium chloride solution) into the left flank 9 days prior to surgery.

All rats were anesthetized during the operative procedures with a combination of halothane and nitrous oxide supplemented by oxygen administered with a close-fitting mask. The respiratory status of the animals was monitored throughout anesthesia, and all surgical procedures were performed under sterile operating conditions. Pneumoperitoneum was achieved using a conventional Veress needle placed through a right hypochondrial stab wound. A disposable small laparoscopy cannula (Imagyn Medical, Laguna Niguel, Calif) was used to provide access for a 2-mm laparoscope (Imagyn Medical) with attached conventional laparoscopy camera. Two additional ports were inserted: an 18-gauge cannula in the left hypochondrium, which was left open throughout the procedure to vent the insufflation gas, and a 16-gauge cannula in the left lower quadrant, which was used to provide access for a needle used for tumor laceration.

After commencing gas insufflation and placing all ports, the tumor capsule and overlying peritoneum were lacerated in a standard fashion under laparoscopic vision using an 18-gauge needle inserted through the 16-gauge cannula, which created direct communication between the tumor and the peritoneal cavity.

Various solutions were introduced into the peritoneal cavity through the 16-gauge cannula immediately after laceration of the tumor. These solutions remained within the peritoneal cavity during the entire period of laparoscopic exposure and following surgery. No attempt was made to aspirate the solutions from the peritoneal cavity.

In group 1, the control rats underwent laparoscopy and laceration of the tumor without instillation of an intraperitoneal agent. In group 2, the rats received 3 mL of 0.9% isotonic sodium chloride solution in the peritoneal cavity. In group 3, the rats received 3 mL of povidone-iodine solution, diluted 1:10 with 0.9% isotonic sodium chloride solution, in the peritoneal cavity. In group 4, methotrexate was introduced into the peritoneal cavity at a dose of 0.5 mg/kg in a solution of 4 mL of 0.15-mol/L isotonic sodium chloride. In group 5, 3 mL of undiluted aqueous chlorhexidine acetate was introduced into the peritoneal cavity. In group 6, methotrexate (0.5 mg/kg) was administered by intramuscular injection 30 minutes before conventional carbon dioxide insufflation commenced. Intraperitoneal agents were not used in this group.

Gas was insufflated at a rate of 0.4 L/min and a pressure of 2 mm Hg for 40 minutes after tumor laceration, with a constant gas flow maintained through the venting cannula. After 40 minutes, all ports were removed, and the puncture sites were closed with sutures. All procedures were performed by a single investigator (S.J.N.). Rats were randomly allocated to treatment groups.

Seven days after the operative procedure the animals were killed, the dimensions of the primary tumor were measured, and the abdominal cavity was opened. The abdominal cavity and the surgical access wounds were examined for the presence of tumor metastasis before excising the port sites for histopathological examination. The presence of intraperitoneal adhesions was also noted, and the rats were examined for tumor involvement of the axillary and femoral lymph node groups. Animals were weighed preoperatively and at the time of autopsy. Excised specimens were fixed in buffered formaldehyde prior to embedding in paraffin for sectioning and subsequent staining with hematoxylin-eosin. All specimens were examined in a blind fashion by a histopathologist (T.D.) who was unaware of the group or the anatomical site of origin of the specimens.

The protocol for this study was approved by the animal ethics committees of the Institute of Medical and Veterinary Science and the University of Adelaide, Adelaide, Australia.

RESULTS

The measured size of the primary tumor at the time of surgery was similar in all study groups, and the growth of the implanted tumor following surgery was similar in all study groups ($P = .42$) (**Table 1**). In addition, the body weight of the rats was similar in all groups (**Table 2**). During autopsy, tumor was visible as a nodule at the site of intraperitoneal tumor laceration in all rats. However, there was no evidence of tumor dissemination to any site other than the surgical wounds. **Table 3** and **Table 4** summarize the development of macroscopic and microscopic metastases at the laparoscopic trocar wounds. Tumor involvement of the surgical wounds did not occur following the intraperitoneal administration of diluted povidone-iodine (Table 2) (Fisher exact test; $P = .04$). In contrast, 41.7%, 33.3%, 33.3%, 16.7%, and 41.7% of the rats in the control, isotonic sodium chloride solution,

Table 1. Tumor Growth Following Laparoscopy*

	Median Size of Tumor, mm (Range) by Group					
	Control	Isotonic Sodium Chloride	Povidone-iodine	Chlorhexidine Acetate	Intraperitoneal Methotrexate	Intramuscular Methotrexate
Surgery	35 (15-47)	30 (15-41)	37 (24-48)	42 (30-57)	20 (21-40)	30 (20-46)
Day 7	55 (45-74)	48 (12-69)	58 (52-72)	59 (48-81)	51 (41-62)	50 (40-60)
Increase, %	63 (30-200)	76 (–20-88)	58 (43-125)	52 (10-127)	81 (28-108)	65 (30-100)

**Analysis of variance test was used to compare percentage increase in maximum tumor size (P = .42).*

Table 2. Postoperative Rat Weight*

	Median Weight, g (Range) by Group					
	Control	Isotonic Sodium Chloride	Povidone-iodine	Chlorhexidine Acetate	Intraperitoneal Methotrexate	Intramuscular Methotrexate
Surgery	225 (196-254)	244 (222-287)	220 (203-255)	225 (178-290)	253 (225-283)	239 (215-276)
Day 7	264 (226-280)	244 (166-330)	267 (225-288)	257 (181-321)	273 (245-328)	265 (233-305)
Change, %	13 (–2 to 23)	7 (–26 to 15)	16 (4 to 31)	16 (–7 to 25)	9 (2 to 16)	9 (3 to 15)

**Analysis of variance was used to compare the percentage change in weight (P = .003). The Dunn test was used to compare the percentage change in weight in the control vs the other groups (P>.05) and the saline group vs the povidone-iodine group.*

Table 3. Rats With Metastases in Trocar Wounds*

	No. of Rats by Group					
	Control	Isotonic Sodium Chloride	Povidone-iodine	Chlorhexidine Acetate	Intraperitoneal Methotrexate	Intramuscular Methotrexate
Macroscopic	1	1	0	2	2	3
Microscopic	5	4	0	4	2	5
P	. . .	>.99	.04	>.99	.37	>.99

**Fisher exact test was used to compare the number of microscopic metastases compared with the control group. There were 12 rats in each group. Ellipses indicate not applicable.*

Table 4. Wounds With Metastatic Tumor Present*

	No. of Wounds by Group					
	Control	Isotonic Sodium Chloride	Povidone-iodine	Chlorhexidine Acetate	Intraperitoneal Methotrexate	Intramuscular Methotrexate
Macroscopic	1	1	0	3	3	3
Microscopic	7	4	0	5	3	5
P	. . .	.51	.01	.75	.31	.75

**There were 36 wounds per group. Fisher exact test was used to compare the number of microscopic metastases compared with the control group. Ellipses indicate not applicable.*

chlorhexidine, intraperitoneal methotrexate, and parenteral methotrexate groups, respectively, developed port-site metastases (Table 2). The results of an analysis of the occurrence of metastases in individual wounds mirrored the findings of a significantly lower risk of wound metastasis following the instillation of intraperitoneal povidone-iodine (Table 4) (*P* = .01). One rat in the isotonic sodium chloride solution group developed bacterial peritonitis. This rat survived to 7 days after surgery and was therefore not excluded from analysis.

The development of metastases to the left axillary lymph nodes occurred in 5 rats in the intraperitoneal methotrexate group and 6 rats in the parenteral methotrexate group (**Table 5**). No other palpable lymph nodes were noted. No metastases were found in either the control group or the povidone-iodine group.

COMMENT

Since 1978 when Döbrönte et al[14] first reported a case of tumor seeding following laparoscopic surgery, it has been recognized that the laparoscopic manipulation of an intra-abdominal malignant neoplasm can be associated with an incidence of metastasis to surgical access

Table 5. Presence of Left Axillary Palpable Lymphadenopathy*

	Control	Isotonic Sodium Chloride	Povidone-iodine	Chlorhexidine Acetate	Intraperitoneal Methotrexate	Intramuscular Methotrexate
Palpable axillary lymph nodes	0	2	0	3	5	6
P	. . .	.48	>.99	.22	.04	.01

**Fisher exact test was used to compare the presence of lymphadenopathy compared with the control group. Ellipses indicate not applicable.*

wounds. However, whether this incidence of metastasis is more likely to occur following laparoscopy than conventional open surgery is controversial.[2] Nevertheless, the results of recent experimental studies[5,15] tend to support those who believe it is a specific problem of minimal access surgery.

Previous studies[16,17] have established that viable cells are liberated into the peritoneal cavity during the resection of malignant tumors, and many authors[5,9,11] believe that in the presence of a pneumoperitoneum these viable cells can be aerosolized, resulting in implantation in the laparoscopic access wounds. Alternatively, cells may implant in wounds due to direct contamination from laparoscopic instruments.[2,4,18] For this reason, some authors[6,19] have proposed the use of intraperitoneal chemotherapy as an adjuvant strategy to decrease the incidence of port-site metastases following laparoscopic surgery.

Intraperitoneal chemotherapy has been used clinically for the treatment of gastrointestinal tract cancers.[7,8] The rationale behind this approach is that administration of a chemotherapeutic agent at a time when optimal killing of viable intraperitoneal cells can occur (eg, immediately following laparoscopic surgery) could possibly reduce the risk of transcoelomic tumor spread due to operative intervention.[6] Tumor cells may be shed as a result of surgical trauma, resulting in the spillage of cells into the operative field, or they could leak indirectly from raw vascular and lymphatic channels.[8] These viable cells may then implant preferentially in raw peritoneal surfaces and sites of peritoneal abrasion, in particular the surgical access wounds.[8,18,19] Wade et al[20] demonstrated that as few as a single viable tumor cell implanting at the port site may result in a metastasis.

It has also been demonstrated that there may be a pharmacological advantage to the intraperitoneal administration of cytotoxic agents at the time of surgery[8] and that this may result in a reduction in the risk of peritoneal spread. This advantage is due to both direct contact between tumor cells and the tumoricidal agent[6] and changes in cell kinetics.[21] The removal of the primary tumor is thought to stimulate the proliferation of noncycling cells, thereby rendering the cells more vulnerable to the effects of cytotoxic agents. Also, the peritoneal clearance of a drug is slower than plasma clearance, prolonging exposure time. Other advantages of intraperitoneal chemotherapy administered at the time of surgery include an even distribution of the agent throughout the peritoneal cavity before the development of any adhesions, concurrent recovery from both the operation and chemotherapy,[22] and the possibility of decreased systemic adverse effects. Methotrexate was chosen for our study because previous work[12] confirmed that the tumor line we used is sensitive to methotrexate and tumor progression can be halted with a 1-mg/kg dose.

Many surgeons[23,24] routinely use peritoneal lavage at the end of surgical procedures involving resection of malignant neoplasms. Intraoperative irrigation using 0.9% isotonic sodium chloride solution may remove some spilled tumor cells. However, since it is not possible to adequately irrigate all peritoneal surfaces,[25] viable tumor cells potentially could remain within the peritoneal cavity. Cytotoxic agents, such as povidone-iodine and aqueous chlorhexidine, are also commonly used as lavage agents during surgery to treat cancer.[23] Povidone-iodine has been demonstrated to be significantly more cytotoxic to breast cancer cells in vitro[26] compared with bleomycin sulfate, hydrogen peroxide, or water. The efficacy of povidone-iodine in killing colon cancer cells in a rat model has been demonstrated by Docherty et al.[23] In the study by Docherty et al,[23] the use of intraluminal povidone-iodine siginificantly reduced the incidence of tumor recurrence while the use of chlorhexidine had no effect.

The results of the present study demonstrate a significant reduction in the incidence of laparoscopy-associated wound metastases following the use of intraperitoneal povidone-iodine. This suggests that the tumoricidal effects of povidone-iodine may reduce the number of viable tumor cells in the peritoneal cavity and prevent the implantation of viable cells into port-site wounds. This effect was not seen with any of the other agents tested. Interestingly, there was no reduction in the incidence of port-site metastases following the administration of either intraperitoneal or parenteral methotrexate. This finding was unexpected. It is possible that the dose used was not enough to produce effective cell killing or it was too much and the rats' systemic immunity was suppressed, thus negating any beneficial effects. The latter explanation seems likely as spread of tumor to lymph nodes was more common following methotrexate administration.

It is possible that the correct dose of an intraperitoneal agent is critical to the success of this modality of treatment. During initial pilot studies, inadvertent administration of a higher dose of intraperitoneal methotrexate resulted in one rat developing increased tumor growth and spread, including malignant ascites. Antimetabolite substances such as methotrexate may require administration of more than 1 dose to affect cells not in the active cell cycle. Previous work[12] demonstrated that a dose of 0.5 mg/kg intramuscularly repeated at 24 hours is required to achieve an antitumor

effect. Similarly, the concentration of povidone-iodine administered was varied in pilot studies to establish a concentration that was not toxic to rats.

In this study, tumor size was unaffected by the agent used. This finding suggests that there has been no or minimal systemic effect of the agent used. The percentage weight gain in the rats was also unaffected by the agent used. This finding suggests that there was no significant systemic toxic effect of the agents on the rat. Left axillary lymph nodes were palpable in significantly more rats in the methotrexate groups, suggesting that there may have been a suppression of the rats' systemic immunity with this agent. The left axillary lymph nodes are nearest to the primary tumor site and are probably involved as the initial draining lymph group.

The results of this study demonstrate that the development of port-site metastases can be reduced by the use of intraperitoneal povidone-iodine. Other strategies were less effective, although this may be a limitation of the dose used. Although these findings warrant further investigation within clinical trials, washing out the abdominal cavity with diluted povidone-iodine should be considered outside such trials in cases of laparoscopic surgery for malignant neoplasms.

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Corresponding author: Susan J. Neuhaus, MBBS, University Department of Surgery, Royal Adelaide Hospital, Adelaide, South Australia, 5000 Australia (e-mail: dwatson@medicine.adelaide.edu.au).

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