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The efficacy of agents employed to prevent anastomotic recurrence in colorectal carcinoma

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Summary

Forty-eight of 72 surgeons canvassed in the South West of England (67%) routinely use an intraluminal cytotoxic agent to prevent suture-line recurrence following partial resection of the large bowel for cancer. The most popular agents are chlorhexidine-cetrimide preparations (n=14), mercuric perchloride (12), povidone-iodine (7) and water (12); noxythiolin, sodium hypochlorite and silver nitrate are used occasionally. The mean duration of treatment is 2 minutes. When assayed for cytotoxity against tumour cells freshly prepared from human colorectal carcinomas (n=10), both chlorhexidine-cetrimide and povidone-iodine were rapidly lethal at a wide range of concentrations (5–100%). Mercuric perchloride (0·2%) was similarly effective, but up to 20% of tumour cells remained viable after exposure to noxythiolin and nearly 30% with water alone. Chlorhexidine-cetrimide and povidone-iodine are the agents of choice to kill malignant cells exfoliated into the colorectal lumen.

Introduction

Implantation of exfoliated cancer cells into freshly cut tissues was postulated by Sir Charles Ryall some 75 years ago *(1,2)*. Later this hypothesis was advanced to explain some suture-line recurrences after partial colectomy for cancer *(3,4)*. To prevent implantation by exfoliated tumour cells various precautions have been recommended, including per-operative irrigation of the intestinal lumen with cytotoxic agents and isolation of the tumour with tapes before handling the bowel. As a result the incidence of suture-line recurrence was reduced *(5–7)*. However, Rosenberg's recent experimental studies indicating that exfoliated colorectal cancer cells are seldom if ever viable appeared to refute the likelihood of implantation recurrence *(8,9)*. To the contrary our preliminary studies suggest that large numbers of viable exfoliated carcinoma cells can be readily retrieved from sites of intestinal transection in patients with large bowel cancer *(10)*. The potential implication of this finding led us to investigate current surgical practice in the prevention of suture-line recurrence and then to assess the efficacy of the main agents employed.

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The Editor would welcome any comments on this paper by readers

TABLE I *Questionnaire*

1. Do you routinely 'clean' the bowel ends with a particular tumouricidal agent before anastomosing the colon or rectum?
2. Which agent or agents do you use?
3. What concentration of agents is used?
4. For how many minutes do you 'clean' the bowel ends?
5. Do you employ any other surgical techniques to minimise suture line recurrence?

Materials and methods

Measures taken to prevent implantation metastasis Ninety surgeons in the South West of England were circulated with a questionnaire (Table I) regarding their current surgical practice in cleansing the cut ends of the bowel before performing an anastomosis during operation for carcinoma of the large intestine.

Preparation of tumour suspension Biopsies (ca 10 g) of 10 colorectal carcinomas were collected fresh at operation. The tumour-bearing resection specimen was immediately opened and washed with tissue culture Medium 199. Fat and necrotic debris were cut from the tumour biopsy, which was finely diced with scissors. Tumour fragments were digested in Medium 199 containing 2·0 mg collagenase/ml (Type 1 from *Clostridium histolyticum*, activity 200 IU/mg, Sigma) and 0·2 mg deoxyribonuclease/ml (Type 1 from bovine pancreas, Sigma). Following incubation with magnetic stirring at 37 °C for 60 min, the resultant cell suspension was filtered through a 60 gauge stainless steel mesh and washed ×3 in Medium 199 at 170 g for 5 min before being resuspended in Medium 199 enriched with 10% v/v foetal calf serum. Centrifugation at 60 g for 10 min produced a tumour-cell-rich pellet, which was resuspended in Medium 199. The number of viable tumour cells was determined by exclusion of the supravital stain trypan blue (0.165% w/v in Medium 199). After mixing for 5 min, counts of unstained tumour cells were performed at a 1 in 10 dilution of tumour suspension in trypan blue using an improved Neubauer haemocytometer. Tumour cells were identified by their characteristic morphology.

Cytotoxicity assay of agents Agents tested were chlorhexidine-cetrimide and povidone-iodine at 5 different concentrations, noxythiolin at 3 concentrations, mercuric perchloride 0.2 % and distilled water (Table II). Medium 199 was the control.

TABLE II *Cytotoxic agents and concentrations tested*

Proprietary name	*Trade name*	*Percentage concentrations tested*
1. Chlorhexidine gluconate (0.015% w/v)-cetrimide (Ph Eur 0.15% w/v)	'Savlodil'	100, 10, 5, 3, 2.5
2. Povidone-iodine 7.5% w/v	'Betadine'	100, 10, 5, 3, 2.5
3. Mercuric perchloride	—	0.2
4. Water	—	—
5. Noxythiolin	'Noxyflex'	5, 2.5, 1

The tumour suspension was adjusted to contain 1×10^6 viable tumour cells/ml. One ml aliquots of suspension were centrifuged at 60 g for 10 min to produce a tumour cell-rich pellet. The pellet was resuspended in 2 ml of the agent under test and incubated for 5 min at 37 °C. Following centrifugation at 170 g for 10 min and resuspension of the pellet in 1 ml of Medium 199, the number of viable tumour cells was determined by trypan blue exclusion (0.165 % w/v).

The median percentage of viable tumour cells after incubation in an agent was compared to that obtained after incubation in Medium 199, and the difference was tested by Wilcoxon's rank sum test.

Results

Surgical practice in the prevention of implantation metastasis Seventy-two of the 90 surgeons who received a questionnaire replied. Forty-eight of the 72 (67 %) routinely treat the bowel ends with a cytotoxic agent for a mean duration of 2 mins (range 30 sec to 20 mins). Various methods of treatment were employed. For distal large bowel tumours rectal irrigation with the agent was performed either before clamping and resection or by distal irrigation of the rectal stump after transection of the bowel; the lumen of the proximal colon was then swabbed with the agent. The agents and the concentrations routinely employed are shown in Table III. Fifteen of the 72 surgeons (21 %) isolate the tumour with tapes before mobilising the bowel.

TABLE III *Agents used to prevent suture line recurrence*

Agent	*Concentrations employed (%)*	*No. of surgeons using agent*
Chlorhexidine-cetrimide	100	9
	30	5
Sterile water	—	12
Mercuric perchloride	0.2	9
	0.1	3
Povidone-iodine	100	5
	20	1
	5	1
Noxythiolin	5	1
Silver nitrate	0.5	1
Sodium hypochlorite	50	1

Cytotoxicity of agents The results of the cytotoxicity assays are shown in the Fig. All agents reduced the number of viable tumour cells when compared to incubation in Medium 199 ($P<0.001$), despite the fact that only two-thirds of cells remain viable after incubation in the control medium. All concentrations of povidone-iodine achieved near-total cell kill, as did the 2 highest concentrations of chlorhexidine-centrimide. At concentrations of less than 10 % chlorhexidine-cetrimide an increasing number of tumour cells survived. Mercuric perchloride 0.2 % was the third most effective agent and considerably more reliable than noxythiolin at any concentration. Water was the least effective agent, with a median of 28.5 % surviving tumour cells.

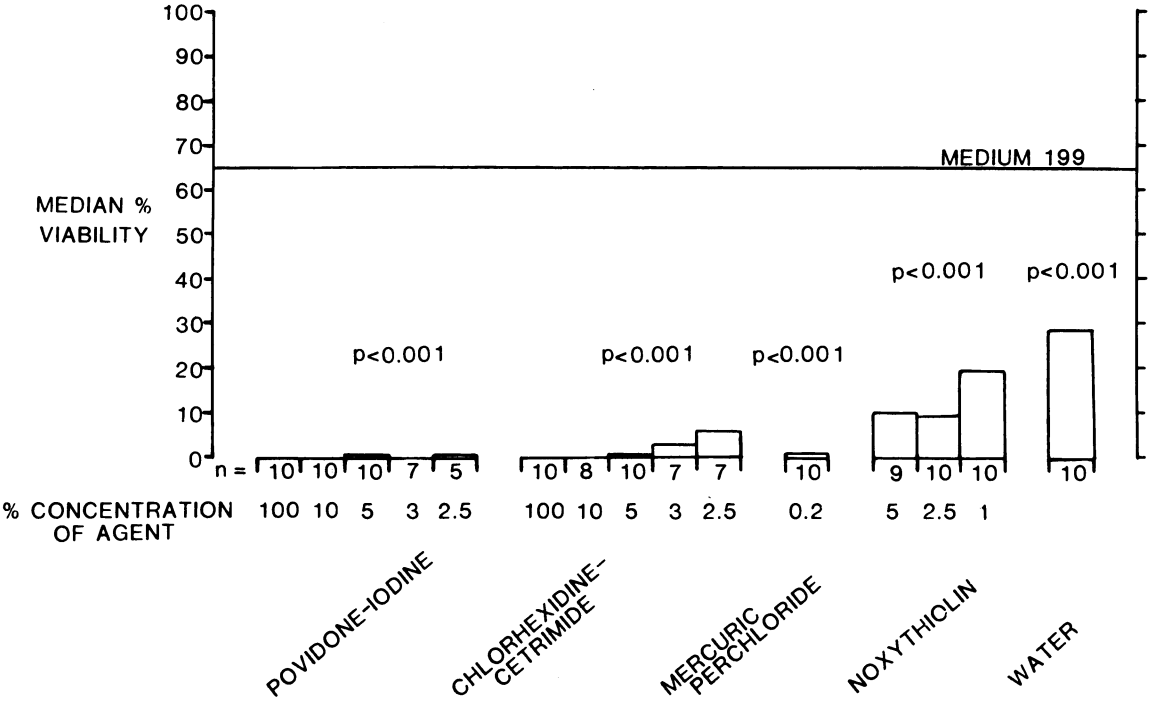

FIG. Median percentage of viable tumour cells after incubation with povidone-iodine, chlorhexidine-cetrimide, mercuric perchloride, noxythiolin and water. The number of assays (n) is shown in the open boxes.

Discussion

As many as one-third of surgeons have abandoned any method of preventing implantation of exfoliated colorectal cancer cells. Presumably they believe that exfoliated cancer cells are a less likely cause of local recurrence than incomplete resection or retrograde lymphatic spread *(11)*. Rosenberg's findings certainly argue against the probability of malignant cells implanting on the anastomosis and would strongly support this explanation *(8,9)*. Yet anastomotic recurrence following radical resection of Dukes' A and B tumours is more difficult to ascribe to residual disease. Alternatively some 'late' recurrences at the suture-line could represent metachronous cancer developing at a site of chronic irritation *(12)*. In support of implantation metastasis is the reported reduction from 10–16 % to 2–3 % in the incidence of suture-line recurrence following the introduction of various preventive measures *(5–7)*. Further clinical support for implantation comes from reports of carcinoma deposits (in association with proximal colonic tumours) occurring on haemorrhoidectomy wounds *(13)* and on anal fissures and fistulas *(14)*. These anecdotal reports suggest that exfoliated tumour cells are potentially capable of implantation and proliferation on raw surfaces such as surgical anastomoses.

Previous in vitro studies using human and mouse tumour cell lines have shown cetrimide and noxythiolin to be cytotoxic *(15–17)*. The mechanism of action of cetrimide and chlorhexidine is uncertain, but the end result is disruption of the cell membrane. Noxythiolin is believed to act by slow release of formaldehyde, which combines with amino groups of enzymes to alter their nature and function *(16)*. Since the release of formaldehyde is slow, a longer period of exposure might result in improved cytotoxicity. Mercuric perchloride has been extensively used with reported clinical success ever since tumour-cell implantation was suggested as a cause of suture-line recurrence *(6)*. Yet animal experiments have not confirmed the clinical experience *(18)*. Mercuric perchloride acts by reversible binding to sulphydryl groups of essential cell enzymes, ultimately leading to cell death. Iodine liberated from povidone-iodine is an irreversible oxidant of essential cellular enzymes and causes rapid cytotoxicity. Our results confirm its remarkable efficacy even in dilute solution. All agents tested demonstrated significant cytotoxicity towards colorectal carcinoma cells. Differences in response are probably the result of different exposure times required for each agent to achieve maximal cytotoxicity. As most surgeons only treat the bowel ends for a short period of time, it would seem sensible to use the most rapidly effective agents such as povidone-iodine or chlorhexidine-cetrimide, at a 10 % concentration or stronger.

Our preliminary data showing large numbers of viable exfoliated colorectal cancer cells at the site of intestinal transection in patients with colorectal carcinoma *(10)* supports the continued use of routine measures to prevent implantation recurrence at the suture-line. If surgeons continue to dispense with such measures, there is a danger that suture-line recurrence could become an increasing clinical problem.

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