Antiseptic toxicity to breast carcinoma in tissue culture: an adjuvant to conservation therapy?

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Of 50 patients who had scrape cytology of the excision cavity after conservative surgery for breast carcinoma, 10 (20%) had malignant cells remaining in the cavity recognised by cytology. Of these patients, 18 had histological evidence of tumour at the resection margin, giving an accuracy of the cytology of 84%, a sensitivity of 56%, and a specificity of 100%.

When assayed for cytotoxicity against a breast tumour cell line (MCF7) or human fibroblasts, chlorhexidine gluconate was the most effective of eight antiseptics or antitumour agents (100% cytotoxicity at a 1/10 000 dilution) in killing breast tumour cells and had 70% toxicity to human fibroblasts at the same dilution. Hydrogen peroxide appeared to be the most useful agent overall with 94% cytotoxicity to breast tumour cells with only a 12% cytotoxicity to human fibroblasts at a dilution of 1/1 000 000.

We suggest that free malignant cells left in the cavity after conservative surgery for breast cancer may be a cause of local recurrence. They can be recognised by scrape cytology at operation and the topical use of antiseptics as cytotoxic agents may be beneficial and warrants further investigation.

The incidence of breast carcinoma in women living in the Western World is rising and remains one of the leading causes of death from cancer (1). Despite many new techniques of surgical or adjuvant treatment, there has been little improvement in the 10-year survival rate over the past 40 years (2). Any investigation which can improve the adequacy of primary treatment is therefore also likely to reduce the mortality and morbidity from this disease. Conservative surgical procedures have become a viable alternative option to mastectomy for many women who have breast cancer (3). This concept is based on serial randomised clinical trials which have shown that survival and local tumour control after breast conservation are comparable to that achieved with more radical procedures (4–7). However, limited surgery cannot ensure that the cancer is adequately excised and this is one argument against conservative treatment, particularly as there are no guidelines for the extent of surrounding normal breast tissue which should be excised around the tumour. We have therefore examined the postexcision breast cavities intraoperatively, using cytology to assess whether excision was complete, in 81 patients undergoing breast lump excision for suspected or confirmed carcinoma.

This preliminary study showed that following conservative surgery malignant cells remained within the excision cavity of some patients, and this finding led us to investigate the use of local cytotoxic adjuvant treatment. We examined the cytotoxic effects of several antiseptic and antitumour agents on a breast tumour cell line (MCF7) and human fibroblasts in tissue culture to determine whether topical intraoperative application of cytotoxic agents might have any therapeutic value before closure of the breast cavity after tumour excision.

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Patients and methods

Intraoperative cytology

A series of 81 patients undergoing breast lump excision for suspected or confirmed carcinomas were assessed. A final diagnosis of carcinoma was made on histological examination in 50 patients. The median age of the ‘malignant’ group was 57 years (range 27–80 years) and that of the ‘benign’ group was 41 years (range 19–75 years). Wooden spatula scrapings were taken from the tumour excision cavity in the breast immediately before skin closure and after haemostasis was secured. Air-dried slides were prepared and a Giemsa stain applied. The slides were then examined for the presence of malignant cells.

Cell culture

Primary human fibroblast cell cultures were initiated from a piece of skin taken from the edge of a biopsy removed from one of our patients. Under sterile conditions, the biopsy was washed with phosphate-buffered saline (PBS) containing 400 U/ml penicillin and 200 µg/ml streptomycin, transferred to a Petri dish and finely minced using a scalpel. The resulting tissue was washed twice with PBS, and transferred to a Universal tube containing 10 ml of 0.1% collagenase and 0.05% trypsin in Earle’s balanced salt solution. The tissue fragments were digested for 20 min at 37°C on a rotator, after which the supernatant was removed, and fresh enzyme solution applied. Following a further 2 h digestion period, the resulting cell suspension was filtered through sterile gauze and 10% (v/v) fetal calf serum added to inhibit further action of trypsin. The cells were spun at 350g for 10 min, the supernatant discarded, and the pellet of cells resuspended in a small amount of culture medium consisting of RPMI 1640 supplemented with 2 mM l-glutamine, 10% fetal calf serum, 200 U/ml penicillin and 100 µg/ml streptomycin. The cells were seeded into tissue culture flasks at a density of 2.5 × 10^4 cells/ml, and incubated at 37°C in a 95% air/5% CO2 humid atmosphere. After 3 days, the cultures were washed with sterile PBS and fresh culture medium applied. When the fibroblasts reached confluency they were deemed suitable for cytotoxicity experiments. MCF7 cells (derived from pleural effusion of human breast carcinoma ATTC HTB22) were a gift from Dr P Moncourrier, Université de Montpelier, France. Cells were maintained in Eagle’s minimum essential medium (MEM) supplemented with 10% fetal calf serum, 1% MEM non-essential amino acids, 2 mM l-glutamine, 200 U/ml penicillin and 100 µg/ml streptomycin.

Both cell types were routinely subcultured and prepared for experimentation using a solution of 0.2% EDTA and 0.05% trypsin (1:250) in PBS.

Cytotoxic agents

Agents tested were: chlorhexidine gluconate 5% w/v; hydrogen peroxide 3%; povidone-iodine 7.5% w/v (Betadine®); chlorhexidine gluconate 0.015% w/v– cetrimide 0.15% w/v (Savolidil®); Eusol substitute 25%; noxythiolin 2.5% (Noxyflex®); mercuric chloride 0.1% and taurolidine (Taurolin®) 2%.

Cytotoxicity assay

MCF7 or human fibroblasts were seeded at a density of 1 × 10^4 into 96-well microtitre plates and grown overnight in the appropriate culture medium. Concentrations of several different aqueous antiseptics ranging from stock to 10^-6 stock solution concentration were prepared using culture medium; 200 µl of each solution was added to the cells and incubated for a further 24 h. A colorimetric cytotoxicity assay was used to measure the number of viable cells remaining after incubation with each antiseptic. Following incubation, the cells were washed, fixed with 5% formalin, and stained with 1% methylene blue in 0.01 M borate buffer (pH 8.5). After rinsing with distilled water, the dye fixed on the residual cells was eluted using 0.1 N hydrochloric acid and the optical density read on a spectrophotometer at 665 nm. The absorbance of the eluted dye is proportional to the number of viable cells present so that the percent-age cytotoxicity is:

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\left(1 - \frac{\text{O.D. cells + antiseptic solution}}{\text{O.D. cells + culture medium}}\right) \times 100
\]

where O.D. is the optical density.

Results

Intraoperative cytology

Of the 50 patients with histologically confirmed carcinoma, 14 (28%) also had evidence of incomplete excision histologically (defined as extension of tumour to the resection margin of the specimens). Of these, 10 had cellular cytology smears adequate for examination, of which seven were malignant. In the remaining three cellular smears benign cells only were found, and one of these patients had a second operation based on the histological findings, but no further malignancy was found in the second specimen. Only 13 (26%) of the patients could therefore be judged to have had an incomplete excision at the first operation. Five further patients (10%) had histological evidence of lymphatic or vascular invasion, despite a histologically complete resection margin. One of these had an inadequate smear and three had malignant smears. One had benign cells only. This gave an accuracy of 84%, a sensitivity of 56% and a specificity of 100%. If the inadequate smears are discounted, the sensitivity was 71%. The inadequate smears were all made early in the study, which suggests that the technique of taking scrapes with the wooden spatula had to be learned by the surgeon performing the operation.
toxicity to MCF7 cells was observed at dilutions greater than 1/1000 from that of the standard stock concentration. In contrast to this, Fig. 2 demonstrates that human fibroblast cultures were not as sensitive as MCF7 cells to low concentrations of antiseptics and thus significant toxicity was primarily seen at dilutions less than 1/1000. The most effective antiseptic was chlorhexidine gluconate which was 100% toxic to MCF7 cells and 95% toxic to human fibroblasts at a 1/10 000 dilution. However, it would appear that hydrogen peroxide and Eusol substitute are more specifically cytotoxic to MCF7 cells than to human fibroblast cultures. When applied at a 1/1000 dilution, Eusol substitute killed 88% of MCF7 cells, but

**Cytotoxicity of antiseptics**

All the antiseptics tested in this study appeared to be toxic to both MCF7 cells and human fibroblast cultures *in vitro*. From the results shown in Fig. 1, significant

**Figure 1. (a, b) Effects of antiseptics on MCF7 breast tumour cells in vitro.**

No patient in the benign group (31) or in the completely excised group with no vascular or lymphatic invasion had a malignant cell smear. When the data were combined, the overall accuracy of the technique was 90% with the same sensitivities and specificities.

**Figure 2. (a, b) The effects of antiseptics on human fibroblasts in vitro.**
only 6% of human fibroblasts. Similarly, hydrogen peroxide at a dilution of 1/1 000 000 was 94% toxic to MCF7 cells but only 12% toxic to human fibroblasts.

Discussion

The reported incidence of local recurrence in recent studies of conservative breast surgery, with adjuvant radiotherapy, varies greatly due to different treatment regimens and patient selection. A NSABP trial reported an 8% local recurrence at 5 years for segmental mastectomy and radiotherapy, but excluded patients with positive histological margins who underwent mastectomy (8). Montague (9) reported a 5% 5-year local recurrence, but more than 10% of patients had in situ disease only. Although there is no evidence that local recurrence influences long-term survival it could nevertheless be more devastating psychologically to the patient than the original diagnosis, and is evidence to her of primary treatment failure. It also necessitates, in many cases, more extensive surgery.

There is no clinical data from controlled trials to support any specific surgical technique, in particular how wide a margin of normal breast tissue should be included around the tumour. The goal of conservative surgery is to achieve local control and inadequacy of surgical excision and intralymphatic extension correlate with the risk of recurrence (10–12). The multicentricity of breast cancer has been much debated as a possible contraindication to breast-conserving therapy. Holland (13) found in consecutive cases that 17% had multifocal disease. Gump (14) in another study found an incidence of 19%. Both are higher than the 10% seen in the NSABP trial (8).

However, the natural history of such subclinical lesions is not known (7) and their prevalence may be overestimated in pathological studies of selected patients. Most recurrences also occur close to the previous excision site after breast conserving treatment which favours the cause of recurrence being due to inadequate local excision.

The technique of intraoperative cytological assessment of the excision cavity may alert the surgeon to incomplete tumour excision at the time of surgery and if more extensive surgery is not undertaken may act as a prognostic index. Adjuvant treatment may be adjusted accordingly. The technique is simple and can produce a result quicker than frozen section. In our opinion and from these early results the sensitivity of the technique might improve with experience.

The possibility of implantation of exfoliated tumour cells into newly cut tissues was postulated by Ryall in 1908 (15). Today various precautions have been recommended in colorectal surgery to prevent implantation of exfoliated tumour cells. These include peroperative irrigation of the intestinal lumen with a cytotoxic agent. The incidence of suture-line recurrence has been shown to be reduced using such precautions (16,17). Umpleby and Williamson have demonstrated that topical chlorhexidine-cetrimide and povidone-iodine have effective cytotoxicity against extraluminal tumour cells shed from colorectal carcinomas (18).

It is accepted that postoperative radiotherapy decreases the rate of local recurrence in breast cancer and that lower doses of radiotherapy result in higher recurrences (19). In this study we have found that hydrogen peroxide and Eusol substitute show a preferential cytotoxic action towards MCF7 (breast tumour) cells than to human fibroblasts when applied at appropriate concentrations in vitro. This suggests that it may be practicable to sterilise the tumour excision bed by local irrigation using a cytotoxic agent in a way analogous to the current practice in colorectal surgery. In this study we have shown that malignant cells are frequently present in the cavities left after conservative surgery for breast carcinoma and can be harvested by scrapings and recognised by cytology. The cytotoxic effects of antiseptics in tissue culture suggest that they may be a useful adjunct to conservation therapy.

References

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Assessor’s comment

This is a very well-designed and executed experimental project. From the in vitro experiment they have shown that hydrogen peroxide and Eusol substitutes show a preferential cytotoxic action towards MCF7 tumour cells and human fibroblasts when applied in appropriate concentrations. They have also harvested tumour cells from the cavity following conservative management of breast cancer. Local antiseptic applied to the cavity would, therefore, be a useful adjuvant to conservative therapy.

However, because of the multicentricity of breast cancer it may be difficult to confer the same benefit in vivo, and hopefully they will now proceed to a controlled, double-blind trial in vivo.

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Notes on books


The intention of this book is to integrate into one volume the experiences of distinguished surgeons in dealing with complicated problems of general surgery. The authors have been selected because of their particular expertise in the problem they discuss. Three-quarters of the book is devoted to surgery of the gastrointestinal tract and includes such problems as short bowel syndrome, low rectal carcinoma, biliary fistula, liver injuries and retained common bile duct stones. There are also sections on vascular surgery, recurrent groin hernia, burns, acute necrotising fasciitis and intra-abdominal sepsis. Each chapter can be read independently of the others. There can be few general surgeons who would not profit and learn from reading this book.


This new edition of a textbook first published 5 years ago continues to have as its goal the dissemination of detailed information regarding the monitoring of the anaesthetised patient and the patient in the Intensive Care Unit. It is designed to be a reference book. All chapters have been updated to reflect advances and new chapters have been added including sections on pulse oximetry, transoesophageal echocardiography and monitoring and patient safety.


This is a handsome book. Produced in large format, it contains a wealth of high-quality colour photographs as well as an extensive text covering all aspects of the diseases, disorders and surgery of the temporomandibular joint. The early part relates to anatomy and surgical access, together with pathology, medical diseases and imaging. The authors then go on to discuss arthroscopy and arthroscopic surgery in some detail, followed by dislocation, ankylosis and the surgery of internal derangement. There follows an extensive section on growth problems and the volume concludes with a section on unusual surgical diseases and disorders.

An important volume that will surely find a place on the shelves of every maxillofacial unit as well as in the private libraries of a large number of maxillofacial surgeons.