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The activity of locally applied cytotoxics to breast cancer cells *in vitro*

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The ability of commonly used operative lavage solutions to destroy breast cancer cells was investigated. The cytotoxicity of solutions of Savlon®, noxythiolin, povidone iodine, hydrogen peroxide, bleomycin and water on two human breast cancer cell lines was measured *in vitro*. Viable cells were determined by ability to exclude trypan blue. Results have been analysed with standard non-parametric tests and demonstrate that all solutions tested significantly ($P < 0.01$) reduced the number of viable cells recovered when compared with a control solution of phosphate buffered saline. Solutions of Savlon, 2.5% noxythiolin and povidone iodine were more effective than the other agents in reducing the number of recovered viable cells.

Despite advances in the management of breast cancer resulting from a rationalisation of treatment, local recurrence of the disease remains a distressing problem. One mechanism by which a tumour may recur at the operative site is due to the peroperative seeding of viable tumour cells. Since this was first proposed, more than a century ago *(1)*, tumour cells have been identified in the wounds, on instruments and on surgeons' gloves at the time of surgery for a variety of malignant conditions *(2–4)*. To reduce the implantation potential of these cells, peroperative wound lavage with cytotoxic agents has been proposed. Many different lavage fluids have been used for this purpose *(3,5–8)*.

In this study the ability of some commonly used lavage fluids to destroy breast cancer cells has been investigated, so that their value during breast cancer surgery may be assessed.

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Methods

Cells

Two cell lines derived from human breast cancers (MDA-MB. 231 and T47D) were grown as monolayers in plastic T-75 flasks in Dulbecco's modified Eagle's medium with 10% heat-inactivated fetal calf serum. Before each experiment the cells were washed with phosphate buffered saline (PBS) to remove non-adherent, non-viable cells. The cells were freshly harvested by trypsinisation and suspended in PBS at a dilution density of 10^5 cells in each 1 ml of solution.

Experimental design

Solutions of chlorhexidine gluconate/cetrimide (Savlon®), noxythiolin, hydrogen peroxide, bleomycin, povidone iodine and distilled water (Table I) were compared with a control of phosphated buffered saline (PBS). In each case the test solution (1 ml) was added to PBS (1 ml), containing 10^5 cells. These were left in

Table I. Solutions tested against MDA-MB 231 and T47D cell lines

*Solution**	*Concentration*
Phosphate buffered saline	
Chlorhexidine/cetrimide (Savlon)	0.25%, 0.5%, 1%, 5%
Noxythiolin	1%, 2.5%
Hydrogen peroxide	1%
Bleomycin	60 mg% (in saline)
Distilled water	–
Povidone iodine	7.5% w/v

* All solutions were reconstituted in sterile water unless specified

contact for 1 min before centrifugation (2500 rpm, 5 min) to produce a cellular pellet. The supernatant was discarded and the cells were washed with PBS (1 ml) and collected by centrifugation as before. The cells were resuspended in trypan blue in PBS (1 ml) for counting. All cell counts were conducted by one observer, unaware of the solution being tested. In each case four fields were counted on a haemocytometer and a mean taken for each. Cells were considered viable by their ability to exclude trypan blue. This was repeated a minimum of five times for each test system.

To ensure that trypan blue accurately assesses cell viability, the experiments were repeated using 2×10^5 T47D cells. These were performed three times for each test solution. After treatment with the test solution, half of the cells were challenged with trypan blue, as outlined above, and the remaining cells were then resuspended in culture medium, seeded in Petri dishes and incubated at 37°C in a 5% CO_2 enriched atmosphere for 10 days. The culture dishes were then washed with PBS to remove non-adherent cells. Thereafter, the dishes were inspected microscopically for any intact cells. These were tested with trypan blue, as before. Cells were judged viable if they remained unstained or showed evidence of birefringence on phase-contrast microscopy.

Statistical methods

The number of viable cells obtained after each experiment were compared in test and control solutions using a Wilcoxon rank sum test. In order to rank the solutions in order of efficacy, each solution was compared with each other again, utilising a Wilcoxon rank test.

Results

Figures 1 and 2 are graphical representations of the total number of cells recovered and the number of viable cells after treatment with each solution. It is clear that the total number of cells recovered in each test system was

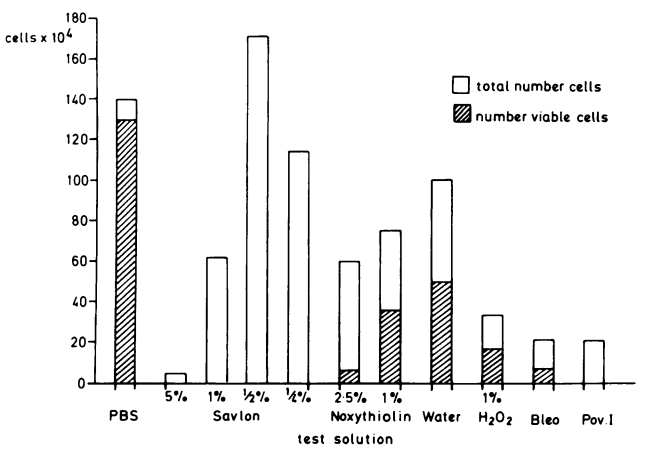

Figure 1. Median number of T47D cells recovered after contact with the test solutions for 1 min. Viable cells are represented by the shaded areas.

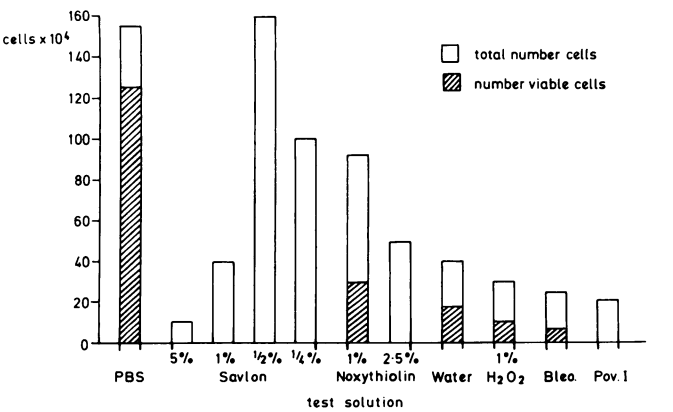

Figure 2. Median number of MDA-MB 231 cells recovered after treatment with the test solutions. Viable cells are represented by the shaded areas.

variable; for example, with the T47D cell line, a mean of 170×10^4 cells were recovered with 0.5% Savlon but only 5×10^4 for 5% Savlon. This pattern of recovery was consistent for the two cell types tested, and although the variation between solutions was marked the variability within each test solution was, in comparison, relatively small (Table II). There was also a qualitative difference between the cells recovered in each of these systems; solutions of 0.5% and 0.25% Savlon caused clumping of the cells, which were non-viable; in contrast, other solutions reduced the number of cells recovered, often with a large amount of cell debris evident. The number of viable cells recovered in each of the systems was reduced compared with the PBS control. This was statistically significant at the $P<0.01$ level for each solution.

Figure 3 shows the order of effectiveness for the solutions tested with the T47D cells. It can be seen that solutions of chlorhexidine/cetrimide (Savlon), 2.5% noxythiolin and povidone iodine can be separated from the other test solutions in order of effectiveness. The MDA-MB 231 cell line was equally sensitive to solutions of chlorhexidine/cetrimide (Savlon) and povidone iodine,

Table II. The number of cells recovered and the standard error of the mean (SEM) after treatment of T47D breast cancer cells with cytotoxic lavage solutions

Solution	*Number of tests*	*Mean No. cells recovered*	*SEM*
PBS	8	168	11.3
5% Savlon	8	3	1.4
1% Savlon	9	41	3.0
0.5% Savlon	6	168	19.6
0.25% Savlon	6	100	4.9
2.5% Noxythiolin	6	50	3.3
1% Noxythiolin	9	71	4.0
Water	6	46	3.3
1% H_2O_2	9	23	4.7
Bleomycin	6	25	2.1
Povidone iodine	6	16	2.1

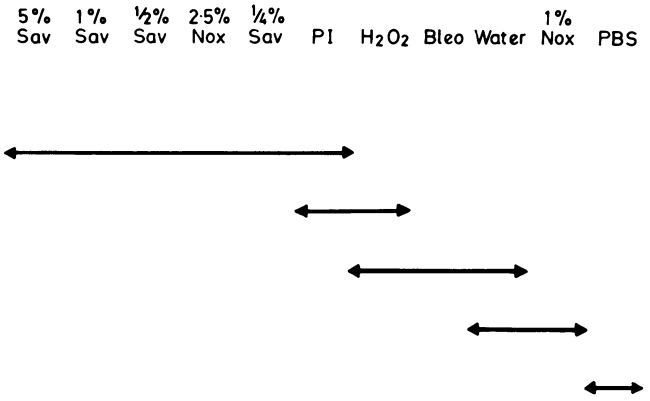

Figure 3. Ranking of the efficacy of the solutions tested against the T47D cell line. Solutions joined by the bars considered equal at the $P > 0.05$ level.

these solutions being the most effective cytotoxics. This cell line, however, was more resistant to 2.5% noxythiolin than the T47D cells, and there was no difference in the observed efficacy between 2.5% noxythiolin, bleomycin and hydrogen peroxide.

In the second series of experiments the initial toxicity assays with trypan blue detected viable cells in the 1% noxythiolin, distilled water, 1% hydrogen peroxide and bleomycin systems. Regrowth experiments, however, identified viable cells only in the PBS and 1% noxythiolin systems.

Discussion

To reduce the potential of exfoliated tumour cells to implant in operative fields peroperative lavage is frequently employed *(3,5–7)*. *In vitro* studies have shown that many lavage solutions are effective cytotoxics *(6,7)*. However, the choice between these has lacked scientific basis. The present study has demonstrated the sensitivity of human breast cancer cells to a variety of commonly used cytotoxic solutions. All of the test solutions significantly reduced the number of viable tumour cells compared with the control solution, and solutions of povidone iodine, and chlorhexidine/cetrimide (Savlon) were the most effective cytotoxics for both cell lines. The failure to regrow any of the tumour cells exposed to these agents, shows that staining with trypan blue is a sensitive indicator of cell death.

The differences in the recovery of cells in each system are likely to represent differences in the mechanism of action of the test solutions. A similar pattern of recovery of cells was apparent with both cell lines. Colonic tumour cells are also killed by a similar range of lavage fluids as were the breast cancer cells in this study, solutions of povidone iodine and chlorhexidine/cetrimide (Savlon) being the most effective cytotoxics *(8)*.

There has been controversy as to the role of exfoliated tumour cells in the development of local tumour recurrence *(9)*. However, in colorectal surgery, methods designed to prevent contamination of the operative field with exfoliated cells, including wound lavage, reduced the local recurrence rate from 10–16% to 2–3% *(10,11)*. Many surgeons already employ lavage of the operative fields in breast cancer surgery or following the biopsy of a suspicious breast lump. Although cell lines, such as those studied, differ in many aspects from the cells released at the time of surgery, it is reasonable to expect a similar range of cytotoxicity *in vivo* as that demonstrated in this study. Any of the solutions tested could, therefore, be used for this purpose, but the results of this study would indicate that with regard to breast cancer, solutions of chlorhexidine/cetrimide (Savlon), and povidone iodine are likely to be the most effective wound lavage. A controlled trial would be required to fully evaluate the effect of peroperative lavages on the incidence of local tumour recurrence in clinical practice. Provided care is taken in patients with a previous history of hypersensitivity, there appears to be little effect of these solutions on wound healing *(6,12,13)*. Systemic toxicity has been reported after the ingestion of large quantities of cetrimide or the repeated cleansing of burns with copious amounts of povidone iodine *(12)*. However, a single peroperative lavage of a wound would not expose the patient to a sufficiently large dose of either agent to cause such problems.

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Notes on books

New Trends in Gastric Cancer edited by P I Reed, M Carboni, B J Johnston and S Guadagni. 192 pages, illustrated. Kluwer Academic Publishers, Dordrecht. 1990. £35.00. ISBN 0 7923 8917 4

The Proceedings of an International Conference held in Rome in 1989. Pathology, diagnostic methods and some aspects of surgical treatment of gastric cancer are covered.

Textbook of Anaesthesia edited by A R Aitkenhead and G Smith. 2nd edition. 773 pages, illustrated, paperback. Churchill Livingstone, Edinburgh. 1990. £32.50. ISBN 0 443 03957 7

The first edition of this book, published five years ago, was well received and had wide sale. The editors have now introduced several new chapters, undertaken major revisions of the chapters on pharmacology and on practical aspects of anaesthesia, and updated the content of the remainder of the volume. A large number of new illustrations are included and many old figures have been revised. A comprehensive, yet concise, readable account of anaesthetic practice which is recommended.

Surgical Pathology of the Breast by K Rogers and A J Coup. 149 pages, illustrated. Wright, London. 1990. £39.50. ISBN 0 7236 0965 9

Abundantly illustrated with a wealth of colour photographs and written jointly by a surgeon and a pathologist, this book should interest many surgeons as well as pathologists, radiotherapists and oncologists. The book brings together physical signs, symptoms and pathology of both benign and malignant disease, together with succinct surgical comment. The text is supplemented by illustrations of gross and microscopic pathology as well as clinical photographs. A useful addition to the literature.

Manual of Pediatric Anesthesia by David J Steward. 3rd edition. 445 pages, paperback. Churchill Livingstone, New York. 1990. £22.50. ISBN 0 443 08573 0

This third edition has been updated throughout and comprises a comprehensive summary of all aspects of paediatric anaesthesia written in short note style. There are no illustrations but key references are given.

The Pediatric Neurosurgical Patient: A Cooperative Approach edited by L P Ivan. 323 pages, illustrated, paperback. Warren H Green, Inc., St Louis. 1989. £38.75. ISBN 0 87527 352 1

This book is particularly directed at health care workers, other than doctors, who are involved with the care of children undergoing neurosurgical procedures. There are chapters on nursing, speech therapy, audiology, psychology, education and rehabilitation as well as on neurosurgery, head injuries and various neurological problems. The necessity of team work in the care of these young patients is stressed throughout.

John Charnley: The Man and the Hip by William Waugh. 268 pages, illustrated. Springer-Verlag, London. 1990. DM75.00. ISBN 3 540 19587 4

The name of John Charnley will be known to every reader of this journal. He was a pioneer and innovator in the field of hip replacement surgery and was the first orthopaedic surgeon to have been elected an FRS. This highly readable biography tells the story of Charnley's single-minded dedication and work in the face of innumerable difficulties. It should prove an inspiration to all young surgeons today as well as a fascinating read for those more senior. The numerous illustrations are also fascinating, being a mixture of the scientific and the personal.

Travels of a Surgeon by Arthur Eyre-Brook. 197 pages, illustrated. White Tree Books, Bristol. 1990. £12.95. ISBN 0 948265 29 9

Many readers of this notice will know personally the author of this fascinating travelogue. A well-known orthopaedic surgeon, Mr Eyre-Brook travelled extensively in the Americas, Africa, Asia and the South-West Pacific. His experiences during these travels are fascinating to read and the book is thoroughly recommended. Although he mentions his surgical activities, most of the chapters comprise comments on the countries he has visited and so the book can be enjoyed by all, whatever their specialty.