# The Prophylactic Treatment of Malignant Disease with Nitrogen Mustard and Triethylenethiophosphoramide (ThioTEPA) *

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IN PREVIOUS publications [3, 4] we have called attention to the danger of implantation of cancer cells into the suture line while resecting the colon for carcinoma, and have advised ligation of the lumen of the bowel above and below the tumor to prevent this complication. However, credit for the first report on the possibility of implantation should go to Morgan and Lloyd-Davies.[13] More recently numerous authors [1, 2, 9, 12, 14] have called attention to local recurrences at the suture line which presumably were caused by implantation. We also have expressed apprehension about the danger of dislodging cancer cells from a tumor during its resection, and have found cancer cells in the blood of a vein leading from the tumor (of the colon) after perfusion with a small amount of saline.[5] To prevent or minimize these venous emboli of cancer cells from reaching the liver we have been ligating all vascular trunks leading to and from the involved area before the resection is started. Fisher and Turnbull [8] have carried this study further by finding cancer cells in the blood of veins (without perfusion) draining from colonic tumors in 32 per cent of 25 specimens studied. In fact, Turnbull [16] reports that in 36 specimens examined when the tumor was handled to an average degree during resection, cancer cells were found in the blood of veins draining these tumors (colon and rectosigmoid) in 28 per cent of cases. In 76 patients having resection without handling of the tumor, he found cancer cells in only 13 per cent.

Perhaps of more significance in the possible spread of carcinoma at the time of operation is the fact that cancer cells can be found in the wound and systemic blood. In a study of 101 major cancer operations Smith and associates [15] demonstrated cancer cells in the "washings" from the wound at the completion of the operation in 34 per cent of cases: in an additional 18 per cent the cells were suspicious. Engel [7] demonstrated cancer cells in the veins draining tumors in various locations in 59 per cent of 107 patients, but more important found cancer cells in the systemic venous blood (antecubital veins) in 4.6 per cent of 65 operable cases and in 50 per cent of 14 inoperable cases.

The rather overwhelming evidence (which has been increasing during the past few years) that cancer cells may be disseminated by operation for cancer has stimulated us to use anticancer agents in a prophylactic way. In a preliminary publication [6] describing work with the Walker 256 carcinosarcoma, we reported that nitrogen mustard appeared to prevent the growth

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TABLE I. *Effect of One Dose (0.5 Mg. per Kg. of Body Weight) of Nitrogen Mustard by One of Three Mechanisms, to Rats Having Cancer Cells Injected into the Portal Vein*

| Group | ℞ Via Portal Vein | | ℞ Via Per. Cavity | | ℞ Via Systemic Vein | |
|---|---|---|---|---|---|---|
| | No. Rats | % "Take" | No. Rats | % "Take" | No. Rats | % "Take" |
| A | | | | | | |
| 110,000 cells | C 48 | 91.7% | C 45 | 86.7% | C 45 | 75.6% |
| Injected | T 45 | 17.8% | T 46 | 41.3% | T 56 | 35.7% |
| | Dif. = 73.9% | | Dif. = 45.4% | | Dif. = 39.9% | |
| B | | | | | | |
| 220,000 cells | C 40 | 82.5% | C 57 | 82.5% | C 35 | 94.3% |
| Injected | C 37 | 27.0% | T 66 | 66.5% | T 38 | 78.9% |
| | Dif. = 55.5% | | Dif. = 16.0% | | Dif. = 15.4% | |
| C | | | | | | |
| Combination | C 88 | 87.5% | C 102 | 84.3% | C 80 | 83.8% |
| of A and B | T 82 | 22.0% | T 112 | 57.1% | T 94 | 53.2% |
| | Dif. = 65.5% | | Dif. = 27.2% | | Dif. = 30.6% | |

C = control, T = treated animals.

of a suspension of cells injected into the portal vein of rats. We chose the injection of a suspension of cells into the portal vein because it would simulate one of the mechanisms of spread of cancer in the human being, i.e., metastasis to the liver. In a more recent publication, we reported data [11] indicating that triethylenethiophosphoramide (thioTEPA) was likewise effective in preventing the take of cancer cells when injected into the portal vein of rats. As reported previously azaserine did not prevent the take in rats injected with a suspension of 110,000 cells as described above.

In the present experiment we have increased the number of animals treated with both drugs, and have made comparative studies utilizing 110,000 and 220,000 cells as the inoculated doses. In addition we are reporting preliminary results when therapy was delayed 48 hours.

## METHODS

We chose the Walker rat 256 carcinosarcoma because it is a hardy tumor and is readily transplanted. Cellular suspensions were prepared by first finely mincing the tumor and adding a small amount of saline as recommended by Lucké and associates.[10] The mixture was then filtered through a fine stainless steel mesh (80 wires per inch), resulting in a filtrate consisting almost entirely of single cells. A cell count was made and the filtrate diluted to the desired concentration (110,000 to 120,000 cells per ml.).

In all the experiments herein reported, the cell suspension was injected into the portal vein of female Sprague Dawley rats weighing between 125 and 175 grams. In one series nitrogen mustard was used in treatment and in the other triethylenethiophosphoramide. Treatment was administered by way of the portal vein, peritoneal cavity, or systemic vein. When the portal vein (actually a major mesenteric vein) was used, treatment was given one minute after injection of the cells but with a different syringe. When either the peritoneal cavity or a systemic vein was used, treatment was injected one hour after injection of the cells.

The rats were inoculated and treated in groups of ten to 20, using every other one as controls. We have learned that the cell suspension should not be older than two

TABLE II. *Effect of One Dose (2.0 Mg. per Kg. of Body Weight) of ThioTEPA Given by One of Three Mechanisms, to Rats Having Cancer Cells Injected into the Portal Vein*

| Group | ℞ Via Portal Vein | | ℞ Via Per. Cavity | | ℞ Via Systemic Vein | |
|---|---|---|---|---|---|---|
| | No. Rats | % "Take" | No. Rats | % "Take" | No. Rats | % "Take" |
| A | | | | | | |
| 110,000 cells | C 30 | 90.0% | C 38 | 92.1% | C 39 | 92.3% |
| Injected | T 28 | 7.1% | T 39 | 17.9% | T 37 | 16.2% |
| | Dif. = 82.9% | | Dif. = 74.2% | | Dif. = 76.1% | |
| B | | | | | | |
| 220,000 cells | C 70 | 61.4% | C 29 | 75.9% | C 38 | 94.7% |
| Injected | T 73 | 24.6% | T 28 | 14.3% | T 39 | 38.4% |
| | Dif. = 36.8% | | Dif. = 61.6% | | Dif. = 56.3% | |
| C | | | | | | |
| Combination | C 100 | 70.0% | C 67 | 82.1% | C 77 | 93.5% |
| of A and B | T 101 | 19.8% | T 67 | 16.4% | T 76 | 27.6% |
| | Dif. = 50.2% | | Dif. = 65.7% | | Dif. = 65.9% | |

C = control, T = treated animals.

hours: if older solutions are used the percentage take will be less. We always injected the "treated" animal with cancer cells before the control so that any decrease in "take" incident to aging of the suspension of cells would be more pronounced in the control animal.

One series of animals was injected with 110,000 cells, whereas another series was injected with 220,000 cells in an effort to determine whether the anticancer agent would be more effective against the smaller dose. Each of these large series was divided into three sub-groups, identified by the route of administration of the anticancer agent, i.e. via portal vein, peritoneal cavity, or systemic vein.

## RESULTS

The results of treatment with nitrogen mustard and thioTEPA on percentage "take" in rats inoculated with a suspension of cancer cells are summarized in Tables I and II. In Table I we noted that following injection of a single dose of nitrogen mustard (0.5 mg. per kg. of body weight), the percentage "take" decreased in all three sub-groups of both the 110,000 and 220,000 cell series. In the first horizontal column (Group A) the results following injection of 110,000 cells into the portal vein are given, and in the second horizontal column (Group B) the results of treatment after injection of 220,000 cells are recorded. Group C represents a summation or combination of A and B. It is realized that the differences noted in each sub-group namely, 73.9 per cent in the rats receiving treatment by way of the portal vein and 45.4 per cent in rats receiving treatment by way of the peritoneal cavity, etc., do not represent true mathematical values for those particular sub-groups. However, we believe it expresses a trend regarding the efficacy of the drug.

In Table II the results in rats inoculated with a suspension of cancer cells into the portal vein and treated with a single dose of thioTEPA (2.0 mg. per kg. of body weight) are recorded. The three vertical columns represent the three methods of administration as described for Table I. Comparison of the data in the two tables indicates that thioTEPA may be slightly more effective than nitrogen mustard. As noted, following treatment with nitrogen mustard,

the drug is less effective in the rats receiving 220,000 cells than in the rats injected with 110,000 cells.

Our preliminary experiments indicate that if treatment is not initiated until 48 hours after inoculation of the cancer cells, the anticancer agents may not be effective. For example, when one dose of nitrogen mustard was given to 25 rats 48 hours after inoculation of 220,000 cancer cells into the portal vein, the incidence of "take" was 96 per cent: in 26 animals acting as controls the percentage "take" was 92 per cent. However, we are enlarging this series which is too small to be decisive.

## DISCUSSION

Our experiments indicate that thioTEPA may be slightly more effective in the prevention of "takes" in rats with the Walker 256 tumor than is nitrogen mustard with the doses used, i.e. 0.5 mg. per kilo for nitrogen mustard, and 2.0 mg. per kilo for thioTEPA. We wish to emphasize again that the difference between the percentage "take" in the control and treated animals cannot be considered a true test of comparative efficiency; likewise, data on the effect of anticancer agents in animals cannot be transferred to the human being. However, perhaps we may assume that data on animal experiments may be considered scout work for the clinician.

Both drugs are less effective when a large dose of cells (220,000) is given to the rats than when 110,000 cells are given. This observation has also been noted by other workers utilizing different drugs and cancer cells. If this observation has true clinical significance it may force us to change our philosophy on the spread of cancer. We have already referred in this presentation to evidence which has accumulated during the past year or so indicating that desquamation or dissemination of cancer cells, particularly during operation, is so profuse that it is obvious not all the cells survive. In other words, because of host immunity or low virulence of the tumor cells, only a small portion of the cells survive and grow into metastases. Using a suspension of Walker 256 tumor cells we have noted that an injection of 1000 cells into the portal vein would yield no "take" in the liver of the animals whereas an injection of 110,000 cells resulted in a "take" in 70 to 100 per cent of animals. Knowing that host immunity is probably an important factor in the growth of these cells into metastases, it would be appropriate, indeed, that we devote more attention to mechanisms which might aid the host in this protective mechanism.

The results of our animal experiments revealing efficiency of anticancer agents in preventing the "take" of cancer cells has stimulated us to give nitrogen mustard to patients on the day of operation to kill loose cancer cells, and perhaps destroy or inhibit microscopical nests of cells ultimately destined to develop into gross metastases, but held in check temporarily by the host. We have chosen carcinoma of the breast, stomach, colon, and rectum because tumors in these areas metastasize by vein as well as by lymphatics. At the present time, we have treated about 45 patients, giving the first of four doses of nitrogen mustard on the day of operation. However, we wish to emphasize that nitrogen mustard is more toxic when given to patients on the day of operation than when given later. This increased toxicity is no doubt related to the bone marrow depression which occurs in practically all patients. We have noted a lowered resistance to infection, and an increased tendency to bleed postoperatively. In this series of 45 patients receiving nitrogen mustard we have had two postoperative deaths. In our opinion, one of these deaths was probably related to the nitrogen mustard therapy, but the other was not. Controls have been set up for these 45 patients; we had adopted the principle of establishing controls by random sequence

in blocks of two in order to eliminate as many variables as possible.

In a small group of animals we have found that nitrogen mustard did not decrease the "takes" in the liver when it was given 48 hours after injection of cancer cells into the portal vein. If these preliminary results prove to be valid in a larger series of animals it would appear that in human beings the maximum safe dose of the anticancer agent should be given at the time of operation. At the present time we divide the total dose of nitrogen msutard, giving one-fourth of it on the day of operation and the rest on three successive days. This method is usually employed in the administration of this drug and should be tolerated better when treatment is started on the day of operation. Unfortunately, considerable time will be required to determine the effect of this therapy, the amount of time varying inversely with the number of cases in the study.

Although we are using nitrogen mustard at the present time in our clinical prophylactic experiments, there is no reason to expect it to be the most effective drug. We already have clinical evidence that one chemical agent can be more effective against a given tumor than another agent. It is highly possible that a screening mechanism utilizing several anticancer agents might be effective in indicating which agent would be the most effective. We are now working on such a screening or sensitivity procedure using tissue culture methods on the cancerous tissue as removed in the operating room. If prophylactic chemotherapy is destined to be successful, such a screening procedure, if effective, would increase its efficiency.

## SUMMARY

Operative removal is the best treatment of cancer. However, evidence has accumulated during the past few years indicating that cancer cells are dislodged or disseminated (into the wound, venous system, and perhaps lymphatics) during this procedure. Although the anticancer drugs introduced during the past few years are not curative when given to patients with advanced cancer, we believe they may be effective in killing "loose" cancer cells which do not have a vascular "root." We also hope that anticancer agents will destroy or subdue the growth of microscopic nests of cells which are temporarily suppressed by host resistance (often for years), but which ultimately give rise to lethal metastases.

When this prophylactic therapy was applied to rats injected with a suspension of Walker 256 carcinosarcoma cells into the portal system nitrogen mustard and thioTEPA were effective in decreasing the percentage of "takes." Only one dose was given, but it was administered on the day of injection of cells. ThioTEPA appears to be slightly more effective in animals than nitrogen mustard. Although the dose was four times greater in the former. Preliminary experiments indicate that these drugs are doubtfully effective when given 48 hours after injection of the cells.

Nitrogen mustard and thioTEPA are more effective in preventing "takes" when given to rats injected with 110,000 cells than when given to rats injected with 220,000 cells. When the dose of cells was reduced to 1000 cells, no "takes" were observed even though no anticancer drugs were given. If recent studies indicating a rather wide dissemination of cells are substantiated, we may assume that this same tolerance to a small dose of cancer cells exists in the human being. This host resistance to a small dose of cancer cells is very similar to host resistance to minor contamination of wounds with bacteria. Host resistance is unquestionably an important factor in the growth of cancer, and should be studied intensely; the key to success in treatment may lie in this phenomenon.

On the basis of the favorable effects of anticancer agents on the "take" of cancer cells in animals, we have begun similar

treatment of human cancer at the time of operation, giving nitrogen mustard on four successive days: the first dose is given on the day of operation. We have treated 45 patients in this manner. Nitrogen mustard is much more toxic when given to patients on the day of operation, so great caution must be exercised in therapy of this type.

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DISCUSSION.—DR. FREDERICK E. KREDEL, Charleston, S. C.: In the pre-antibiotic days we used to have a very meticulous ritual whenever there was a chance of contamination of the operative field by bacteria. I think we have been perhaps less careful concerning cancer cells in the wound. The whole development of exfoliative cytology for diagnosis has directed our thinking toward this possibility, and Dr. Cole's paper is very pertinent. We have been using a dilute solution of nitrogen mustard, 10 mgm. in 500 cc., to irrigate the field of operation in certain cancer cases.

(Slide) In two instances skin grafts were applied after irrigation with nitrogen mustard. This is a photograph of a patient several weeks after mastectomy for rather extensive carcinoma with the application of skin grafts. The wound healed per primam and the skin grafts had a 100 per cent take. We have also successfully applied skin grafts directly over the periosteum of the skull after nitrogen mustard irrigation.

DR. WARREN H. COLE, Chicago, Ill. (closing): Regarding the use of these agents in the human being, I was indeed glad to have Dr. Kredel present the local use in the wound. We have confined the use of the drug locally to the peritoneal cavity, using nitrogen mustard after resection of the colon and rectum. We assume the drug will kill the cells disseminated at the time of operation, and also cells dislodged from the tumor and carried to the liver as emboli during the operation. I think Dr. Kredel's suggestion is a very good one and should be used, particularly in radical neck surgery.