Response of a "Resistant" Plasmacytoma to Alkylating Agents and X-Ray in Combination with the "Excision Repair Inhibitors Caffeine and Chloroquine* (34119)

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One of the frustrating aspects of tumor therapy has been the resistance developed by tumors to the agents used therapeutically. Alkylating agents and ionizing radiation, which are used frequently in tumor therapy at the present time, are designed to cause damage to the DNA of tumor cells such that they can no longer divide. However, it has been demonstrated that both bacterial (1–4) and mammalian cells (5–8) have the ability to repair such damage to their DNA by means of specific enzymic mechanisms.

Mammalian cell lines have been isolated which show resistance to specific alkylating agents. After the development of this resistance, these cells show a cross resistance to other types of alkylating agents and X-rays with little or no change in their response to such antimetabolites as amethopterin, mercaptopurine, florodeoxyuridine, and azaserine (9). This suggests that a common mechanism may be responsible for the resistance shown by tumors toward alkylating agents and ionizing radiation. Wheeler and Alexander (10) have shown that cytoxan-resistant and sensitive tumors implanted bilaterally in hamsters show the same extent of incorporation of the alkylating agents, cytoxan, nitrogen mustard, and thioTEPA into their DNA fractions when these drugs are administered systemically. This work indicates that the DNA of resistant tumors is just as vulnerable as that of sensitive tumors. Despite the fact that the same amount of damage has been done to the genetic material of both types of cells, one cell line continues to grow almost unimpeded while the other cell line dies. In view of the known presence of repair enzymes in mammalian cells, it was of considerable interest to determine the effect of repair inhibitors on the response of tumors treated with alkylating agents or X-rays.

Others have found that the repair mechanism in mammalian cells can be inhibited by caffeine, theophyline, and proflavine, caffeine being the most effective of these inhibitors (11). Work in this laboratory indicates that chloroquine is an effective inhibitor of repair enzymes in bacteria (12) and in normal human lymphocytes.[2] It is the object of the work reported here to show that the repair inhibitors chloroquine and caffeine are effective in rendering cytoxan-resistant plasmacytomas sensitive to the action of cytoxan, nitrogen mustard, and X-rays. These plasmacytomas are examples of tumors which show a very high degree of resistance to alkylating agents. Chloroquine and caffeine are especially suitable for such experiments because of their very low toxicity (13).

Methods. The experiments reported here were conducted using cytoxan-sensitive and cytoxan-resistant plasmacytomas kindly donated by Dr. Glynn Wheeler of Southern Research Institute. The tumors were implanted subcutaneously high on the back or rib cage of golden syrian hamsters. After a lag period of about 12 days, the tumors entered a phase of rapid growth, and were allowed to grow to a sufficient size to assure that they were no longer in the lag phase. This was done to avoid interpreting a continuation of the lag phase as being a positive response to the drugs. They were then treated with one of the alkylating agents in-

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jected intraperitonally, or by X-rays. X-ray treatments were performed because of the known cross resistance and also in an attempt to eliminate the possibility that the positive effects noted might be due to the combined systemic administration of two nonspecific debilitating drugs. The tumors were implanted high on the backs of the hamsters where they could be pulled gently away from the animals in a loose flap of skin. The hamsters were anesthetized, the tumor pulled out from the body of the animal, and an X-ray cone lowered over the tumor in such a manner that the tumor received the bulk of the X-ray dose. This avoided subjecting the animal to a large dose of whole body irradiation. A single X-ray dose of 800 r was used in these experiments. At the time of administration of the alkylating agent or X-ray treatment, some of the animals were given chloroquine injections or drinking water containing 1% caffeine. Those animals receiving caffeine had their drinking water removed the night before the start of the experiment and then returned with caffeine added immediately after treatment with an alkylating agent or X-rays. Tumor sizes were then followed daily after the start of these treatments according to the method of Wheeler and Alexander (10).

Results. The response of these plasmacytomas to nitrogen mustard quite clearly illustrates the problems involved in treating resistant tumors. The resistant tumors, unlike their sensitive counterparts, continued to grow with little change in the growth rate or ultimate size when treated with nitrogen mustard, cytoxan, or X-rays alone. In contrast, as demonstrated in Fig. 1, the growth rate of the resistant tumor was markedly slowed by administration of caffeine or chloroquine in conjunction with the nitrogen mustard. These drugs, when used alone, usually had little or no effect on the growth rate and ultimate size of the tumors. Similar results were noted when cytoxan was used as the alkylating agent (Fig. 2). Again there was little or no change in tumor growth rate when cytoxan was used alone. However, there was slowing of this growth rate when caffeine and/or chloroquine were used along with the cytoxan.

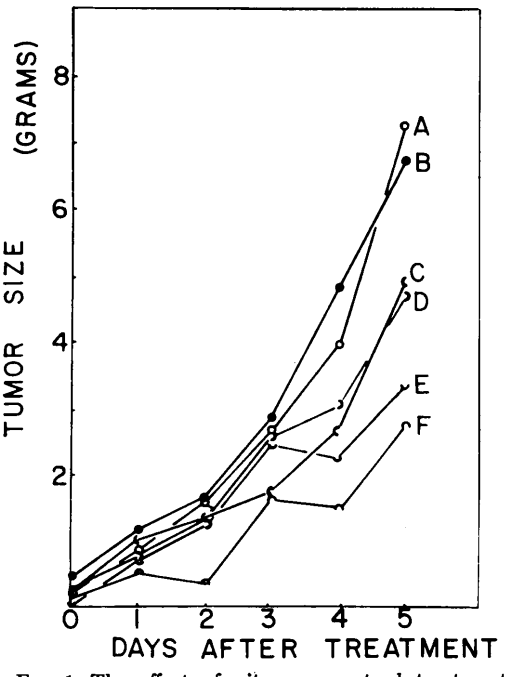

FIG. 1. The effect of nitrogen mustard treatment in conjunction with inhibitors of DNA repair on the growth rates of cytoxan-resistant plasmacytomas. A, untreated controls; B, growth of the resistant tumors in hamsters given 1% caffeine in their drinking water; C, resistant tumors treated with nitrogen mustard alone (1 mg/kg on each of 4 days); D, resistant tumors treated with chloroquine alone (29 mg/kg); E, tumors treated with caffeine plus nitrogen mustard; F, tumors treated with a combination of nitrogen mustard plus chloroquine, 29 mg/kg. Each point on these curves represents the average size of six tumors in grams.

The results were far more clear-cut when single large doses of cytoxan were administered instead of small daily injections (Fig. 3). This greater effectiveness observed when single doses were used is consistent with previous observations. In this series of experiments not only was there a decrease in growth rate but a substantial regression in tumor size 2–3 days after treatment.

These experiments were extended to the use of X-rays.[3] Figure 4 shows that a single

[3] The authors gratefully acknowledge the assistance and advice given by Dr. Robert Roth, Chairman, Department of Radiology, University of Alabama Medical School, which was important to the successful completion of these experiments.

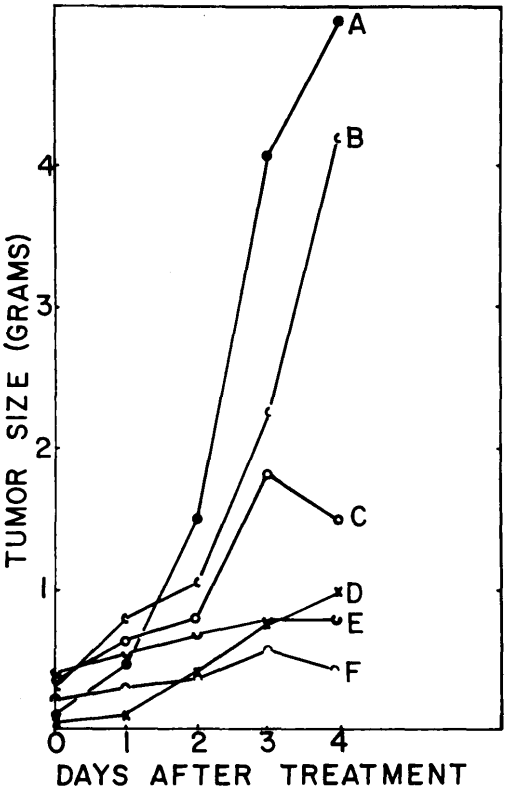

FIG. 2. The effect of cytoxan treatment on cytoxan-resistant plasmacytomas when caffeine and chloroquine are used in conjunction with the alkylating agent. A, resistant tumors treated with cytoxan alone (10 mg/kg on each of 4 days); B, untreated controls; C, chloroquine alone, 29 mg/kg; D, caffeine alone; E, caffeine plus cytoxan; F, chloroquine plus cytoxan.

dose of X-ray failed to halt tumor progression while X-ray in conjunction with chloroquine and/or caffeine resulted in tumor regression.

Delaying the administration of caffeine or chloroquine for 24 hr after the cytoxan or X-ray irradiation eliminated the beneficial effects of the repair inhibitor.

Larger doses of cytoxan (40 mg/kg) caused a temporary slowing of the resistant plasmacytoma's growth rate. This temporary lag was followed by a resumption of the normal growth rate (Fig. 5). Administration of chloroquine or caffeine in conjunction with the cytoxan prevented the usual resumption of growth for at least 4 weeks, the longest time period studied to date.

Similar results have been obtained with alkylating agent- and X-ray-resistant hamster melanoma, and will be reported elsewhere.

Discussion. The results presented here give support to our proposal that resistance shown by tumors to alkylating agents may results from an active repair process and that such resistance can be overcome by inhibiting the repair mechanism of the tumor cells with selected drugs. This rationale provides us with an approach to the treatment of tumors which are presently untreatable because of their high degree of resistance. Obviously this work does not provide direct evidence for the implication of repair in tumor resistance. However, the fact that known inhibitors of repair render resistant tumors sensi-

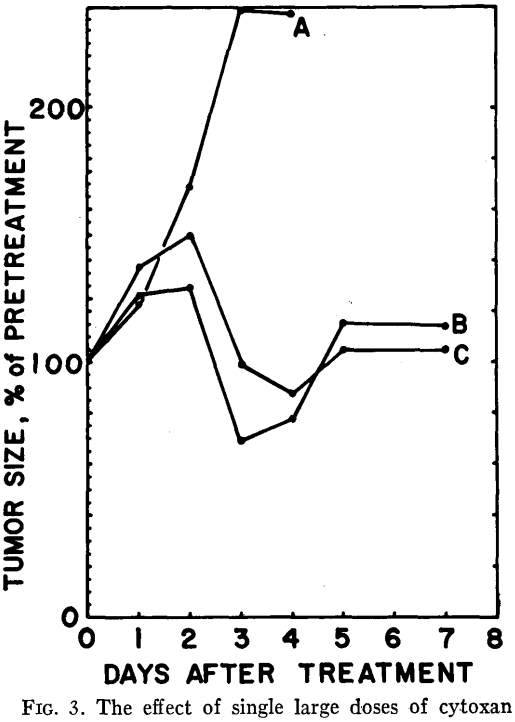

FIG. 3. The effect of single large doses of cytoxan in conjunction with caffeine and chloroquine on growth of cytoxan-resistant plasmacytomas. A, cytoxan alone 25 mg/kg; B, cytoxan plus chloroquine 29 mg/kg; C, cytoxan plus caffeine (1% in drinking water). Each point represents the average size of six tumors within that group.

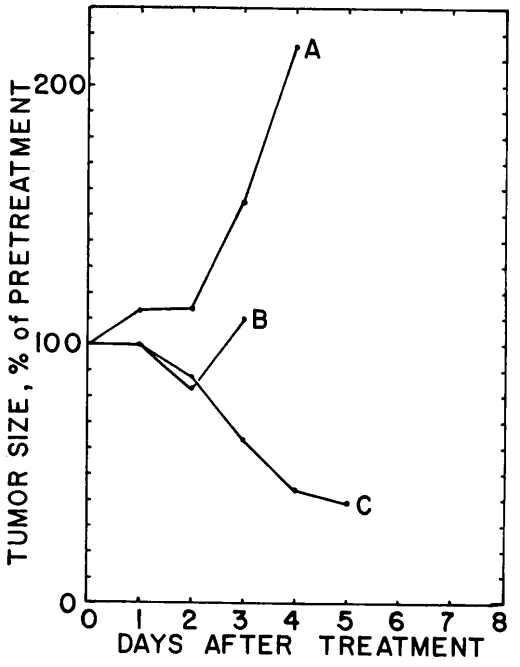

FIG. 4. The effect of repair inhibitors when used in conjunction with X-ray treatment (800 r). A, X-ray alone; B, X-rays plus caffeine (1% in drinking water); C, X-rays plus both caffeine and chloroquine (29 mg/kg).

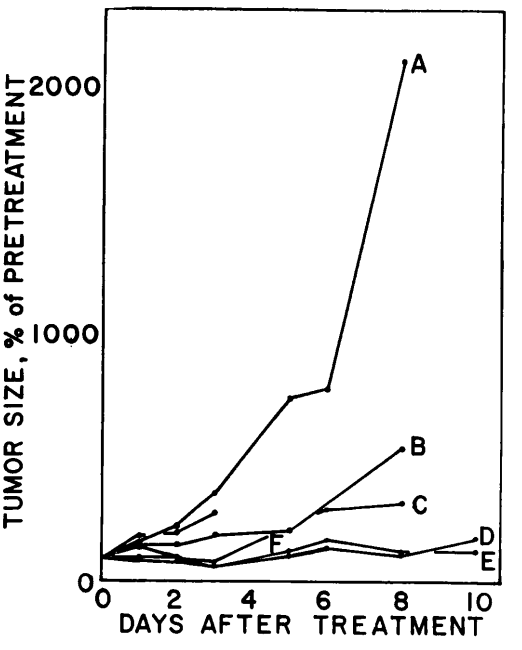

FIG. 5. The effect of single large cytoxan doses (40 mg/kg). Curve A, chloroquine alone; B, cytoxan alone; C, cytoxan plus chloroquine (29 mg/kg); D, cytoxan plus caffeine (1% in drinking water); E, cytoxan plus both caffeine and chloroquine; F, caffeine alone.

tive to the action of cytoxan and nitrogen mustard as well as X-rays, coupled with the known presence of repair in mammalian tissues, makes the explanation advanced above appear reasonable. Earlier work with mammalian cells in culture suggest that repair of DNA damage is accomplished within a few hours (5, 11). Therefore, the observation that a delay in administration of the repair inhibitor is not accompanied by a cessation of tumor growth suggests that the effects noted in the other experiments are not due to a nonspecific debilitating effect of the drugs. Even were this not the case, the effectiveness of this combination of drugs in stopping growth of this tumor is of considerable interest.

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