The Effect of DNA Repair Inhibitors on the Response of Tumors Treated with X-Ray and Alkylating Agents[1] (35544)

DAVID GAUDIN, K. L. YIELDING,[2] AUBRY STABLER, AND JOHN BROWN[3]
(Introduced by J. M. McKibbin)
University of Alabama in Birmingham, the Medical Center, Molecular Biology, Birmingham, Alabama 35233

In recent years, numerous workers have demonstrated that both bacterial (1–4) and mammalian cells (5–7) have the ability to repair a variety of damaging events to their cellular DNA. The alkylating agents and X-rays used in the therapy of malignant diseases are thought to exert their cytotoxic effect in cells principally as a result of chemical alteration of DNA in the affected cells (8, 9), of precisely the kinds which can be repaired by the DNA repair mechanism (8–12). DNA repair, therefore, may play an important role in the resistance expressed by many tumors towards these agents. Inhibitors of this process greatly enhance the cytotoxic effect of alkylation and radiation damage in *in vitro* experiments (13–15). Because of this fact, it was of great interest to determine the effect of the repair inhibitors on the response of tumors *in vivo* to treatment with X-rays and alkylating agents. Earlier work performed in this laboratory has demonstrated that the repair inhibitors, caffeine and chloroquine, when used in conjunction with Cytoxan or X-rays, were effective in stopping the growth of Cytoxan-resistant plasmacytomas under conditions where the alkylating agents or X-rays alone were ineffective (16).

Because chloroquine binds to melanin (17) and is therefore concentrated in pigmented cells, it was also of interest to determine the effect of this DNA repair inhibitor on the resistance to alkylating agents and X-rays shown by malignant melanomas implanted in golden Syrian hamsters. The experiments reported below demonstrate that the use of chloroquine, in combination with either phenylalanine mustard or X-rays, can stop the growth of malignant melanomas implanted in hamsters; whereas neither drug nor X-ray alone at the usual therapeutic doses, is able to stop such growth. Similar results are reported for the use of caffeine in combination with X-rays.

The Cytoxan-resistant plasmacytomas used in earlier experiments were derived from sensitive tumors by treatment with sublethal amounts of Cytoxan. Combined therapy was therefore used with these sensitive tumors in the present experiments, and found to be more effective than the use of Cytoxan alone.

Because the alkylating agents act to supress the hematopoetic system, we have also studied the effect of combined treatment on the white blood counts and bone marrows of mice following combined treatment with chloroquine and Cytoxan.

Materials and Methods. Melanomas and Cytoxan-sensitive plasmacytomas used in these experiments were kindly donated by Dr. Glynn Wheeler of the Southern Research Institute in Birmingham, Alabama. Characterization of the plasmacytomas in terms of their uptake of alkylating agents has been reported by Wheeler (18). Phenylalanine mustard was obtained from Burroughs Wellcome Company as "Alkeran" tablets and was dissolved in sterile saline for injection. Cyclophosphamide (Cytoxan) from Mead Johnson was obtained in vials of 200 mg with 90 mg of sodium chloride and dissolved in 10 ml of sterile distilled water. Chloroquine phosphate (Winthrop Laboratories) was dissolved

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in saline, adjusted to pH 7.4, and then was sterilized by autoclaving prior to injection. All injections were made intraperitoneally. Caffeine (Eastman Organic Chemicals) at a concentration of 1% was administered to the hamsters in their drinking water.

Trocar implants of the tumors were made 10 to 14 days prior to the start of the drug treatment. This period of time was chosen to ensure that all animals used in these experiments had palpable growing tumors. The tumors were implanted high on the rib cage or backs of the hamsters to facilitate measurement of the tumor size. Measurements of the tumors were made immediately prior to the start of the injections and continued at daily intervals for the duration of the experiment. Melanoma bearing hamsters were anesthetized with ether prior to X-ray irradiation.

For a study of the white blood counts, 24 mice were treated with 40 mg/kg of Cytoxan and then were divided into two groups, one of which received an additional injection of 30 mg/kg of chloroquine. Blood from a tail vein was then drawn at daily intervals for the determination of the white blood counts. Bone marrow smears from both femurs of each mouse were made using identically treated groups of animals. The femurs were taken 3 days following treatment of the mice with Cytoxan or Cytoxan plus chloroquine.

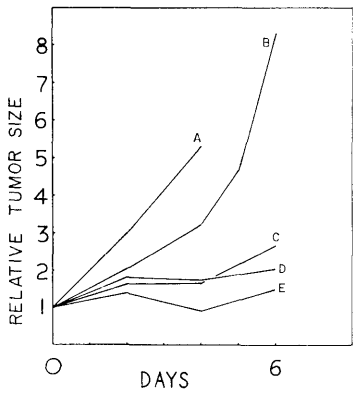

FIG. 1. Effects of the repair inhibitors, chloroquine and caffeine, on the response of melanomas treated with X-rays (800 R): (A) untreated tumors; (B) X-ray only; (C) X-ray + chloroquine (30 mg/kg) daily; (D) X.ray + 1% caffeine in the drinking water; and (E) X-rays + caffeine + chloroquine. Each point on this and subsequent growth curves represents the average of eight tumors.

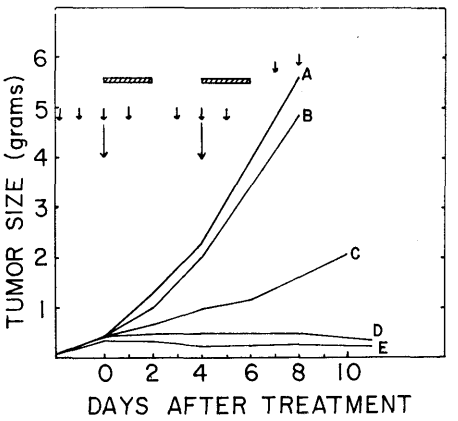

FIG. 2. Effects of chloroquine and caffeine on the response of tumors treated with phenylalanine mustard (3 mg/kg) at times indicated by the large arrows: (A) untreated tumors; (B) chloroquine treatment alone at times indicated by small arrows; (C) phenylalanine mustard (3 mg/kg) alone; (D) phenylalanine mustard + chloroquine (30 mg/kg); (E) phenylalanine mustard + chloroquine + caffeine (1% in drinking water during time indicated by hatched areas).

Results. The results obtained upon treatment of the malignant melanomas with X-ray alone (Fig. 1) clearly illustrate one of the major problems of tumor therapy, namely the high degree of resistance shown by this type of tumor to treatment with X-rays.

In Fig. 1, the size of the tumors is expressed relative to the average tumor size at the start of the treatment period. Actual tumor sizes are shown in Fig. 2. Each point on the graphs of tumor size represents the average weight of eight tumors. After a brief lag in the growth curve following irradiation of the malanomas with 800 R the tumor growth resumed with no intervening regression in size. By way of contrast, the use of caffeine or of chloroquine in conjunction with the X-rays resulted in a cessation of growth and in some cases a regression in tumor size. This effect of caffeine and chloroquine is consistent with previous observations using Cytoxan-resistant plasmacytomas (16).

A similar result was seen when the malanotic melanomas were treated with phenylalanine mustard (Fig. 2). When used at a dose of 3 mg/kg, the phenylalanine mustard had a marked slowing effect on the tumor growth.

Growth of these tumors could not be stopped or caused to regress at levels of phenylalanine mustard which were not fatal to the hamster. However, when chloroquine was used in conjunction with the phenylalanine mustard, there was cessation of the melanomas growth and frequently a regression in tumor size. Curve B in Fig. 2 shows quite clearly that chloroquine when used alone had little or no effect on the growth of these tumors.

In order to observe an effect of the DNA repair inhibitors on Cytoxan-sensitive plasmacytomas treated with Cytoxan, it was necessary to use a suboptimal dose of the alkylating agent of 6.5 mg/kg, which just slowed the growth of the tumors to about one half that of the untreated controls, (Fig. 3). When chloroquine was used in conjunction with this treatment, there was complete cessation of growth followed by regression in tumor size. Repeated doses of Cytoxan alone at this dose level ultimately resulted in tumor regression as well. As has been demonstrated with other tumors, the use of chloroquine alone had little or no effect on tumor growth.

Determination of the white blood counts of mice following treatment of these animals with either Cytoxan alone or in combination with chloroquine produced a surprising result in that the addition of chloroquine to the

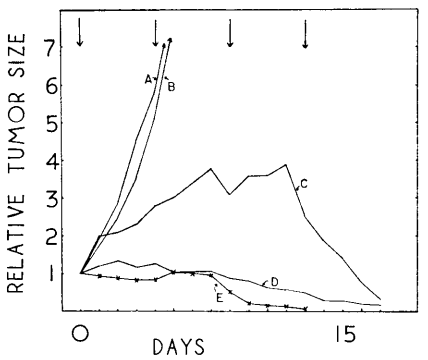

FIG. 3. Effects of chloroquine or caffeine on the response of Cytoxan sensitive plasmacytomas treated with suboptimal doses of Cytoxan: (A) the growth of untreated controls; (B) treatment with chloroquine alone (30 mg/kg); (C) Cytoxan alone (6.5 mg/kg at times indicated with arrows); (D) Cytoxan + chloroquine; (E) Cytoxan + caffeine (1% in drinking water).

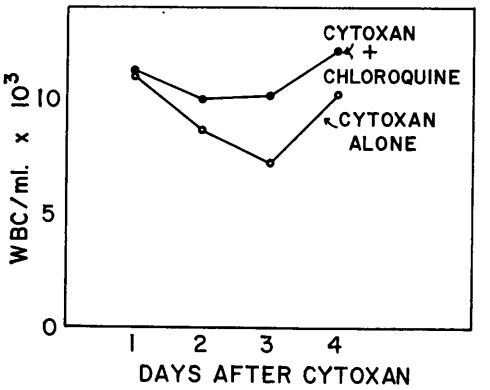

FIG. 4. Effects of Cytoxan (40 mg/kg) and of Cytoxan + chloroquine (30 mg/kg) on the peripheral white counts of mice: each point represents the average from 12 mice. There is no significant difference between the two curves.

Cytoxan treatment did not result in a greater lowering of the white blood count (Fig. 4). The white counts on these curves do not differ significantly. Examination of bone marrow smears made 3 days after combined chloroquine–Cytoxan treatment of the mice showed that the marrows were still highly cellular and that this treatment had not drastically increased the cytotoxic effect of Cytoxan on hematopoetic tissue. The white blood counts from these animals typically reached a minimum 3 days following Cytoxan administration and then returned to normal after a slight rebound to higher levels. Differential white counts performed at 3 days did not show any difference between the two groups. More extensive studies of the toxicity of combined therapy have been performed and will be reported in a separate paper.

Discussion. As indicated in the introduction, the principal reason for studying effects of DNA repair inhibitors on tumors treated with alkylating agent or X-rays lies in the fact that these cytotoxic agents interact with and cause the type of damage to cellular DNA which can be repaired by the DNA repair mechanism (8–12). Caffeine and chloroquine have been chosen for this study because they are repair inhibitors which are relatively nontoxic (19). Also, a great deal of clinical experience has been obtained with the use of chloroquine as an antimalarial. The ability of

chloroquine to bind to DNA (20, 21), probably accounts for its ability to inhibit the repair process. Similarly, numerous other compounds which bind to DNA also inhibit DNA repair (22). Chloroquine has a number of inhibitory properties, including inhibition of DNA and RNA polymerases (23), aminoacylation of some but not all tRNA's (24), DNAase (21), electron transport (25), and protein synthesis in brain mitochondria.[4] DNA repair, of course, includes both nuclease and polymerase activities. As shown in the preceding results, caffeine and chloroquine enhance the effect of alkylating agents and X-rays as measured by the response of otherwise highly resistant tumors.

The Cytoxan-resistant plasmacytomas used in previous experiments (16) were derived from sensitive tumors. It seems reasonable to suggest, therefore, that the factor or factors conferring resistance to the resistant forms of the tumors might also be present in the sensitive tumors but at a lower level of activity. This concept is supported by the evidence showing that the repair inhibitors used render these tumors even more sensitive to Cytoxan. Thus, the use of these inhibitors may be of use with sensitive as well as resistant tumors. Although the effect of combined therapy on tumor growth is what one would expect from the use of a DNA repair inhibitor, the observation that it did not increase toxicity, as shown by the white blood counts and bone marrow smears, was surprising, and may have important implications in chemotherapy. In light of the fact that chloroquine also appears to protect normal skin against the damaging effects of X-rays (26), this drug would seem to have a differential or selective effect on various reactions involving DNA both in different cell types and within a given cell type. For example, chloroquine inhibits replication of the bacteriophage ϕX-174 at concentrations which do not inhibit growth of the host cell (27). It also inhibits growth of the malaria parasite without a deleterious effect on the human host, and in the experiments reported here was able to enhance the effect of alkylating agents and X-rays without having a noticeable effect on the growth of tumors when used alone. This latter observation would suggest that DNA synthesis in the tumor cells was unimpaired by chloroquine alone. Clearly, any speculations involving differential effects of chloroquine in chemotherapy are highly tentative and such studies should be approached with caution, pending accumulation of substantial data with subhuman as well as human subjects.

The free radical trapping properties of melanin may contribute to the resistance of the melanomas to X-ray (28, 29). Whether chloroquine affects this property by binding to melanin is not known. Wheeler and Alexander (18) reported that Cytoxan-resistant and -sensitive tumors incorporated C^{14}-labeled Cytoxan into their DNA to about the same extent. Others have observed that resistant forms of Ehrlich ascites tumor cells had developed a relative permeability barrier to nitrogen mustard, thereby decreasing the effectiveness of a given dose of the mustard (30–33). These cells were resistant to concentrations of mustard which, on the average, would be expected to alkylate every gene in the cell despite the presence of the permeability barrier. Under such circumstances, the permeability barrier could not account for the high resistance of these cells. A very active repair process, on the other hand, can account for such a high resistance (30), as well as the cross resistance to multiple types of alkylating agents (34), and the cross resistance to X-rays as seen in the experiments reported here.

These experiments showing the ability of the DNA repair inhibitors to increase the sensitivity of tumor cells to alkylating agents and to X-rays without having an apparent large increase in toxicity to hematopoetic tissue may provide a new approach to the treatment of tumors with these agents. For whatever reason they exert this effect, the ability of this combination of drugs to stop the growth of melanomas and other resistant tumors is an interesting phenomenon worthy of further study.

[4] Dr. William Bridgers, University of Alabama Medical Center in Birmingham, personal communication.

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