ENHANCEMENT OF THE RADIATION RESPONSE OF CULTURED TUMOR CELLS BY CHLOROQUINE

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The treatment of tumor cells in culture with chloroquine after irradiation significantly reduces their survival, but no enhancement is seen when the cells are treated with the drug before irradiation. This suggests that the potentiation of the radiation response by chloroquine results from the impairment of the post-irradiation recovery processes. Incorporation studies with melanotic melanoma cells in culture derived from human malignant melanoma demonstrate significantly higher uptake of the compound than in amelanotic melanoma, HeLa, or nonpigmented human diploid cells in culture. The significance of these findings is discussed in relation to the potential use of the drug as a radiosensitizer in selected human tumors.

IN THE ABSENCE OF SIGNIFICANT INTRINSIC differential radiosensitivity between most of malignant cells and the surrounding normal cells, attempts have been made to improve the therapeutic index (the ratio of the normal tissue tolerance dose to the tumor lethal dose) by other means. Two such methods are modification of the radiation response of the target tissue with chemicals or, if the tumor cells are partially anoxic, the use of radiation of high linear energy transfer (LET). An optimum means for chemical modification of the radiation effects in clinical radiotherapy would be the use of a nontoxic compound that would be preferentially incorporated into the tumor cells and that would potentiate the lethal effects of radiation, thus enhancing the radiation effects in the irradiated tumor relative to normal cells.

Chloroquine, a well-known synthetic antimalarial compound, has been shown to preferentially enter cells of human malignant melanoma.[1] This compound has also been reported to be an effective inhibitor of repair enzymes in bacteria.[6] Studies with transplantable animal tumors have demonstrated that chloroquine may indeed potentiate the radiation effect in vivo when the compound is administered after irradiation.[3] We have undertaken the present experiments to elucidate the effect of chloroquine on the survival of irradiated cells of several tumor cell lines. We found that melanotic melanoma cells in culture derived from both human and mouse malignant melanoma exhibited significantly higher uptake of the compound than did non-pigmented tumor cells. The human pigmented cells incorporated significantly more chloroquine than did cells from a line (WI-38) derived from a normal human embryo. Furthermore, an appreciable enhancement of the radiation effect was demonstrated in the cells exposed to chloroquine after irradiation. These results suggest that the enhancement of the radiation response by chloroquine is due to the impairment of post-irradiation recovery processes.

MATERIALS AND METHODS

Experiments were carried out with several human cell lines and one mouse line, derived from either malignant tumor or normal tissue (Table 1). All the cultures were grown and maintained in Eagle's minimum essential me-

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dium supplemented with 10% fetal calf serum. Details of the maintenance of the culture procedures have been described elsewhere.[4]

Determinations of the uptake of ^{14}C-chloroquine into the trichloroacetic acid insoluble fractions of the cells were carried out as follows: Equal numbers of cells (approximately 5×10^5 cells per plate) were labeled for 6 hours with ^{14}C-chloroquine (0.1 mCi/ml). Cells were then washed three times with pre-warmed saline and harvested by trypsinization. The trypsinized cells were filtered onto millipore filters and washed with cold 5% trichloroacetic acid. The radioactivity on the filters was determined with a liquid scintillation spectrometer using standard procedures.[5]

To determine the effect of the drug or irradiation on cell viability, the cells from the exponentially growing stock cultures were trypsinized and plated in 60-mm plastic Petri dishes containing 5 ml of the culture media. After incubating for 20 to 24 hours, the drug in 5-10x concentrated stock solutions in saline was introduced directly into plates, which were then incubated for various periods of time. The number of cells plated was selected so that after drug treatment and/or irradiation, they formed 100 to 300 colonies per plate at the end of 2 weeks of incubation at 38C in a humidified chamber with 5% CO_2 and 95% atmospheric air. The number of colonies formed was enumerated after staining with 2% crystal violet.

Irradiations were performed with a Picker x-ray unit, and irradiation parameters were as follows: 280 Kvp, 20 ma, HVL 0.5 mm Cu; dose rate 154 rads/min. All irradiations were performed at room temperature.

RESULTS

Uptake of ^{14}C-chloroquine by various human cells in culture: Table 1 shows a significantly higher uptake of ^{14}C-chloroquine into the acid insoluble fraction by melanotic melanoma cells than by non-pigmented human cell lines. Although the incorporation into mouse melanoma cells was only moderately greater than into human WI-38 cells, it is probably more relevant to compare uptake into pigmented and non-pigmented cells of a single species.The rate of incorporation does not appear to be correlated with growth rate. Relative uptake by the different cell lines was similar for 6-hours and 24-hours incubation periods.

TABLE 1. Incorporation of ^{14}C-Choloroquine by Cultured Cell Lines

Cell line	Doubling Time (hours)	CPM/10^6 cells $\times 10^{-3}$
Human malignant melanotic melanoma (SK MEL-1)	150	39.6 (38–41)
Human malignant amelanotic melanoma (HT 144)	120	4.3 (3.3–5.3)
Human embryonic lung fibroblast (WI 38)	24	13 (12–14)
Human epidermoid carcinoma of cervix (HeLa S-3)	20	3.3 (3.0–3.6)
Mouse malignant melanoma (B-16)	24	19.5 (21–18)

Equal numbers of cultured cells were incubated with ^{14}C-chloroquine (0.1 μCi/ml) for six hours. Cells were then harvested, and the radioactivity in the acid-insoluble fractions were determined as described in reference (5). Values shown are the means and ranges of values from two separate experiments in each system.

Pre- and post-irradiation treatment of HeLa cells with chloroquine: A series of experiments with HeLa cells treated with the drug either before or after irradiation was carried out in order to study the temporal relationships of the radiation-enhancing effect of chloroquine on the treated cells. Figure 1 shows a pronounced potentiation of the cells treated with chloroquine after irradiation, while the treatment of cells with chloroquine before irradiation causes no appreciable increase in lethality, at least for incubation periods of less than 12 hours (Fig. 2). Figure 3 shows the survival curves of HeLa cells exposed to 50 μg/ml for 6 hours before or after irradiation. It is quite evident that the post-irradiation treatment with the drug is more effective in reducing the cell viability than is the pre-irradiation treatment.

The fact that the enhancing effect of chloroquine in irradiated cells is manifested with post-irradiation drug treatment strongly suggests that the drug interferes with a recovery process of the irradiated cells. Figure 4 shows the temporal relationship governing the interference of the recovery process by the drug. This inhibition of the recovery process disappears rather promptly, and survival reaches a

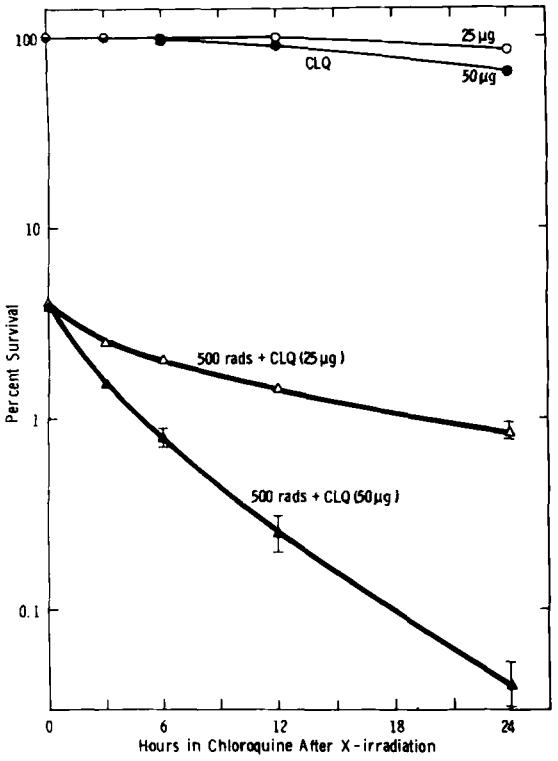

FIG. 1. Post-irradiation treatment of HeLa cells with chloroquine as a function of time after a single dose of 500 rads (lower curves). The upper curves show survivals with chloroquine only (no irradiation). Each point represents the mean of six experimental values (three replicates from each of two separate experiments). Standard errors of the mean are indicated by error bars, except when less than the diameter of the corresponding data symbol. Plating efficiency of the controls (no drug treatment or irradiation) was 60%.

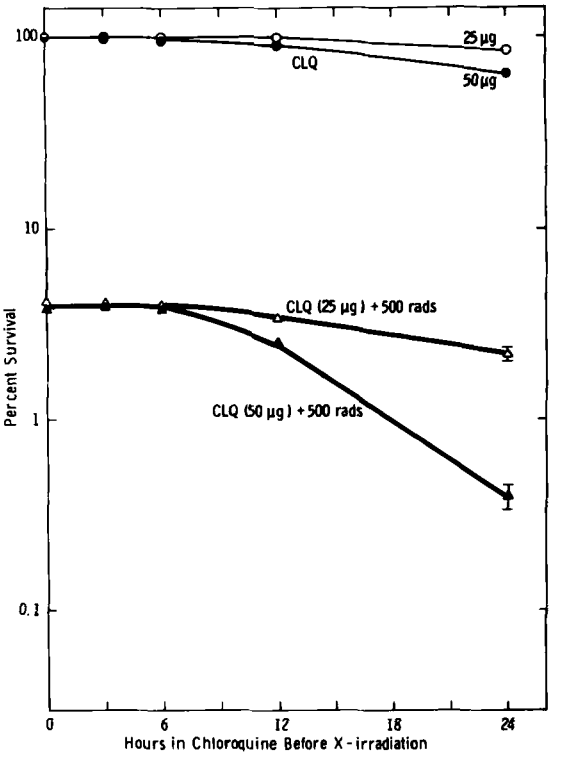

FIG. 2. Pre-irradiation treatment of Hela cells with chloroquine as a function of time before a single dose of 500 rads (lower curves). Upper curves show survivals with chloroquine only (no irradiation). See legend to Fig. 1.

maximum of 80% of the control within 6 hours after irradiation.

Enhancement of the radiation effect of mouse melanotic melanoma cells by chloroquine: Because of the low plating efficiency of human malignant melanotic melanoma cells in culture, an experiment was carried out with mouse melanotic melanoma cells in culture instead. A similar pattern of potentiating effect of chloroquine on this cell line was obtained (Fig. 5). It is worth noting that the viability of the mouse melanoma cells was severely affected at the concentration of 10 µg/ml, which has no appreciable effect on HeLa cells, a non-pigmented human cell line. Significant ehancement of the radiation effect was observed only at a concentration of 5 µg/ml or above.

DISCUSSION

Chloroquine has been shown, at least in our cell culture systems, to meet the basic requisite

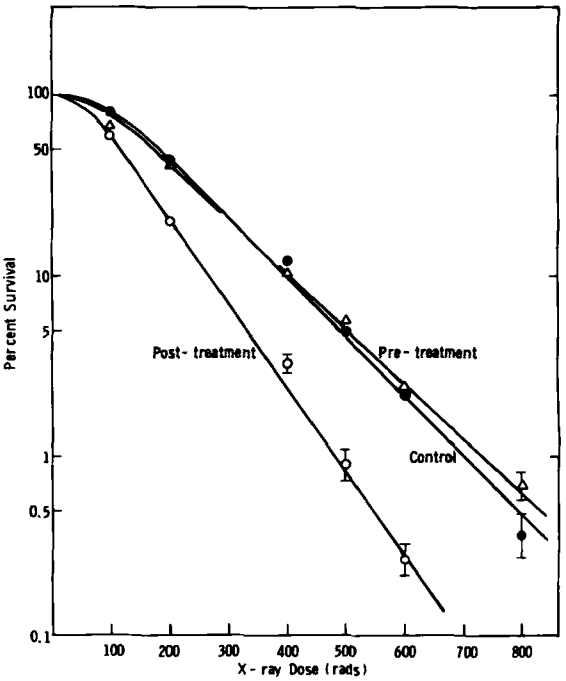

FIG. 3. Pre- or post-irradiation treatment of HeLa cells with chloroquine (50 µg/ml) for 6 hours. See legend to Fig. 1.

of a radiosensitizing agent for use of clinical radiotherapy, i.e., preferential uptake by (melanotic) tumor cells (Table 1), resulting in lower drug-induced toxicity to normal tissues, and potentiation of the radiation effect in drug-treated cells (Fig. 1). The magnitude of this potentiating effect is clearly dependent on the drug concentration of the medium as well as on the duration of exposure of the cells to the compound. Although quantitative cell survival studies could not be carried out with human malignant melanoma cells, a similar enhancing effect of chloroquine on radiation lethality was shown with mouse melanoma cells in culture (Fig. 5). Since the uptake studies demonstrated significantly higher uptake of chloroquine into human malignant melanotic melanoma cells in culture than into amelanotic melanoma or non-pigmented human diploid cells in culture, it is reasonable to anticipate that similar potentiation by chloroquine of the radiation lethality on human melanotic cells would occur. Recently, Beierwaltes et al. have proposed the use of chloroquine labelled with radioiodine as a scanning agent for detection of metastatic malignant melanoma, based on their experience with transplantable malignant melanoma in mice and hamsters.[1]

Little is known about the mechanism of action of chloroquine on the irradiated cells. Chloroquine, like quinacrine, belongs to a group of intercalating agents but is far less toxic and better tolerated than the latter. It has been shown in bacterial systems that the treatment of irradiated *E. coli* K-12 with quinacrine markedly increases the killing induced by irradiation; compound apparently acts as a potent inhibitor of the repair of DNA single strand breaks.[2] It remains to be seen whether the repair of DNA strand breaks is similarly impaired in mammalian cells treated by chloroquine.

Our study with HeLa cells strongly suggests that the potentiating effect of chloroquine in the irradiated cells results from an impairment of the recovery process, since only the post-irradiation treatment with chloroquine results in enhancement of the lethal effect of the irradiation (Fig. 3). Furthermore, the interference with the post-irradiation recovery process by chloroquine lasts for only a short time (Fig. 4). This means that for maximum effectiveness, the peak concentration of chloroquine in the plasma should be reached soon after irradiation and should be maintained at

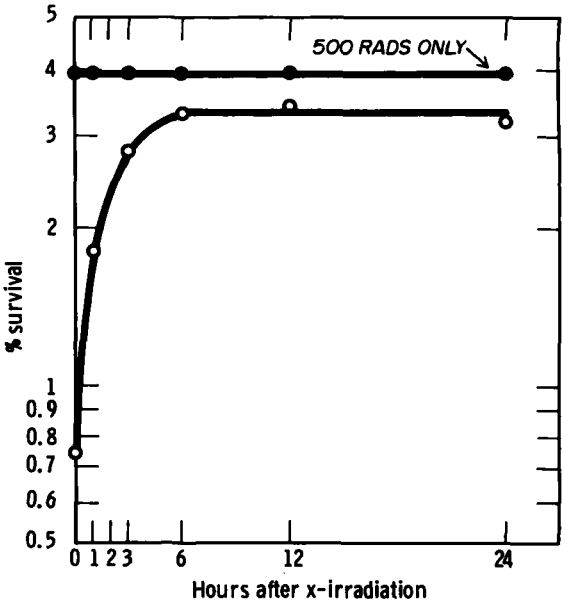

FIG. 4. Effect of delay prior to the addition of chloroquine to irradiated HeLa cells. Chloroquine (50 μg/ml) was added at different times after exposure of the cells to 500 rads; cells were incubated with the drug for 12 hours.

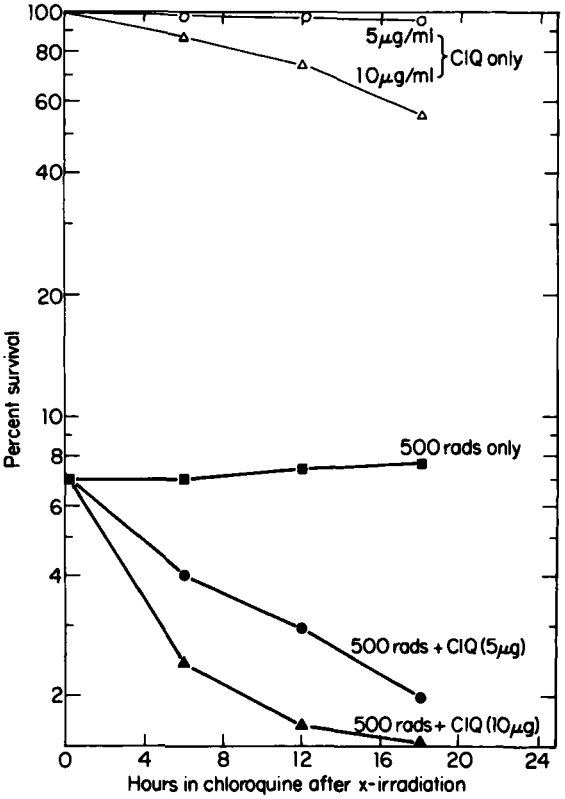

FIG. 5. Post-irradiation treatment of mouse malignant melanoma cells with chloroquine as a function of time after a single dose of 500 rads.

high levels for at least 6 to 8 hours post-irradiation. Obviously, more animal tumor studies are needed to more adequately establish the optimal temporal relationships between chloroquine ingestion and fractionated irradiation treatments.

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