Selective enhancement of cellular oxidative stress by chloroquine: implications for the treatment of glioblastoma multiforme

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✓Chloroquine is used in the treatment of malaria, a disease caused by infection with the parasite *Plasmodium*. Although chloroquine appears to possess diverse pharmacological activity, its plasmodicidal activity results from augmentation of parasite oxidative stress. Chloroquine also appears to augment oxidative stress in metabolically active mammalian cells, including human astroglial cells. The authors propose that chloroquine may augment oxidative stress induced by radiotherapy in the treatment of glioblastoma multiforme, enhancing therapeutic efficacy. Such an effect would be consistent with the known pharmacological effects of chloroquine observed in *Plasmodium*. Other selective redox agents, such as tempol and artemisinin, should be investigated clinically for therapeutic benefit when coadministered with combined radio- and chemotherapy for cancer.

KEY WORDS • glioblastoma multiforme • oxidative stress • chloroquine

GLIOMASTOMA multiforme is the most common and aggressive primary glial tumor, comprising 30% of all brain malignancies in adults.³⁷ This is a rapidly infiltrating tumor that is resistant to current therapies, resulting in a median survival duration of approximately 1 year.³⁷ Despite all efforts, few therapeutic advances have been achieved over the last decade that have significantly improved survival rates. Nevertheless, results from two small studies support the suggestion that the addition of chloroquine to conventional therapy may improve the median survival duration in patients with GBM.^{2,38}

Advances in Treatment of GBM

In an initial study, a statistically significant increase in survival time was found following conventional therapy plus chloroquine (33 ± 5 months) relative to conventional therapy plus placebo (11 ± 2 months, nine patients in each group).² In a second follow-up study, the median survival time increased from 11 months following placebo plus standard therapy to 24 months following chloroquine plus standard therapy (15 patients in each group), and although these results failed to achieve statistical significance, there was a trend toward an increase in overall survival for the chloroquine treatment arm.³⁸ At least two the-

ories have been proposed to account for the apparent therapeutic benefit associated with chloroquine treatment: 1) chloroquine possesses antimutagenic properties that impede the development of treatment-resistant clones; and 2) the lysosomotropic effects of chloroquine lead to cell necrosis.^{2,46} However, an alternative hypothesis, enhancement of cellular oxidative stress, may better explain the apparent improvement in median survival duration noted in patients undergoing concurrent chloroquine therapy.

Importance of Oxidative Stress

The treatment of patients with GBM typically includes surgical tumor ablation and radiotherapy with or without chemotherapy. Postoperative radiotherapy has been recognized as the standard of care for several decades, whereas the additional use of chemotherapy has been controversial.²⁵ Recently, tumor ablation followed by radiotherapy and concurrent temozolomide administration has become the new standard of care.³ In the studies mentioned earlier (Briceno et al. and Sotelo et al.), chloroquine therapy was initiated 5 days postsurgery, prior to the start of radiotherapy (total dose 6000 Gy) or chemotherapy (four cycles of carmustine, 200 mg/m²) in patients with GBM.^{2,38}

In tissues exposed to treatment, radiotherapy produces cytotoxic free radicals and extensive oxidative stress.^{6,8} Ionizing radiation produces a number of reactive oxygen species, including superoxide ion, hydroxyl radical, and hydrogen peroxide; it is currently postulated that the pri-

Abbreviation used in this paper: GBM = glioblastoma multiforme.

mary therapeutic effects from radiotherapy are mediated through the generation of cytotoxic reactive oxygen species.^{6,22} Furthermore, cancer cells accumulate iron, and radiotherapy elevates intracellular free iron concentrations that can lead to the production of cytotoxic hydroxyl radicals through the Fenton reaction.^{19,41} Therefore, cells exposed to radiotherapy are subjected to heightened oxidative stress. These cells must either adapt to such conditions or perish. Adaptation often includes induction of biochemical pathways that quench or mediate the destructive effects of reactive intermediates. In U251 human glioblastoma cells, radiation has been shown to induce the expression of a number of antioxidant enzymes, including superoxide dismutase (twofold) and glutathione peroxidase (fivefold).²² Antioxidant adaptations noted in U251 human glioblastoma cells are consistent with clinical observations of GBM resistance to radiotherapy.^{2,22}

Chloroquine Enhances Oxidative Stress

Chloroquine, a 9-aminoquinoline synthesized in 1934, is used in the treatment of malaria, a disease caused by infection with the parasite *Plasmodium*. Although chloroquine appears to possess diverse pharmacological activities, its plasmodicidal effect results from augmentation of oxidative stress in this parasite.^{10,13,26} During schizogony, *Plasmodium* subsists on amino acids derived from hemoglobin, resulting in production of the prooxidant heme iron complex ferriprotoporphyrin IX. This heme iron complex is detoxified in *Plasmodium* through a number of glutathione-dependent pathways (including incorporation into hemozoin).¹³ Chloroquine appears to inhibit the detoxification of ferriprotoporphyrin IX, in part by diminishing the availability of reduced glutathione.^{7,13} Excess iron and ferriprotoporphyrin IX, in the absence of sufficient glutathione, leads to autooxidation and parasite death. Furthermore, chloroquine appears to interact directly with free ferriprotoporphyrin IX in an electron transfer reaction that produces highly reactive radicals.²⁶ Chloroquine also appears to augment oxidative stress in mammalian cells that have high metabolic activity, and it generates reactive oxygen species in human astroglial cells.^{1,21,27,31,32,40,42}

Therapeutic Benefit of Chloroquine: Potential Mechanisms

We propose that chloroquine may augment oxidative stress induced by radiotherapy in cancer cells. Such an effect would be consistent with the known pharmacological effects of chloroquine in *Plasmodium*.²⁶ Directed radiotherapy selectively produces oxidative stress in targeted cancer cells, generating free radicals and elevating concentrations of free iron.⁴¹ Following such treatment, cancer cells often induce antioxidant defenses as an adaptive response to thwart cytotoxicity. Agents such as chloroquine that promote oxidative stress may hinder the effectiveness of these antioxidant adaptations, resulting in heightened tumoricidal activity. The therapeutic benefit noted with chloroquine appears to be the result of effective proliferation of reactive intermediates initiated by radiotherapy, resulting in increased tumor cytotoxicity.

At least two alternative theories have been proposed to explain the apparent benefit of adding chloroquine to traditional GBM therapy. One theory contends that chloro-

quine is antimutagenic and prevents the development of treatment-resistant clones.² However, there are at least two inconsistencies with this theory. First, there is little evidence that chloroquine is antimutagenic. Although quinacrine, a compound structurally related to chloroquine, has demonstrated antimutagenic properties in two cell lines, it appears to be mutagenic in others.^{12,15,44} Furthermore, most data on chloroquine support its mutagenic properties rather than antimutagenic effects, which is consistent with other 4-aminoquinolines.^{4,9,29,44} Second, if chloroquine possessed antimutagenic properties, diminished rather than enhanced efficacy might be expected with concurrent radio- and chemotherapy, because treatment-induced mutations are what yield the desired tumor cytotoxicity.

Another theory states that the radiosensitization produced by chloroquine is due to its lysosomotropic effects.⁴⁶ The lysosomotropic effects produced by chloroquine, a cationic amphipathic drug, result from protonation and subsequent molecular "trapping" within acidic lysosomes, which elevates lysosomal pH. However, there are vast numbers of cationic amphipathic drugs that have not been reported to induce radiosensitization (Table 1). The radiosensitizing effects of chloroquine appear to be unique relative to other lysosomotropic compounds like ammonium chloride, fluoxetine, and amitriptyline. Although lysosomal membrane rupture contributes to cell necrosis after radiation and chloroquine treatment, the cause of membrane rupture may be lysosomal membrane lipid peroxidation produced by the distinct prooxidant properties of chloroquine rather than lysosomotropism.^{24,30,39}

Discussion

Cancer cells, including malignant glial cells, accumulate iron to a greater extent than do normal cells, producing a condition similar to that found in *Plasmodium*.^{19,28} After radiotherapy, protein-bound intracellular iron is released as free iron, and chloroquine may promote oxidative stress through propagation of reactive oxygen species in a manner similar to the stress produced in *Plasmodium*. Of interest, the antimalarial agent artemisinin has recently demonstrated activity against human glioma and breast cancer cell lines in vitro.¹⁸ Artemisinin, an endoperoxide, appears to be activated to release reactive oxygen species by elevated concentrations of intracellular free iron in both *Plasmodium* and cancer cell lines.^{17,20} Another redox-active compound, tempol (a piperidine nitroxide), demonstrates antiproliferative effects in C6 glioma cells in vitro and in mouse xenografts.¹¹ Tempol, a superoxide dismutase mimetic, converts superoxide into hydrogen peroxide, which in the presence of free iron is converted to the highly reactive hydroxide ion.⁵ Like chloroquine, tempol has demonstrated plasmodicidal activity in vitro, further supporting theories about its role as a prooxidant agent in the presence of available iron.³⁶ Tempol, artemisinin, and chloroquine appear to demonstrate little cytotoxicity in the absence of preexisting oxidative stress, thus sparing normal cells.^{11,34}

Because it appears that chloroquine serves as a promoter rather than an initiator of oxidative stress, it may also prove beneficial in cases in which oxidative stress is ther-

TABLE 1

Selected cationic amphipathic drugs/lysosomotropic agents not associated with radio- or chemosensitization

Agent	Pharmacological Class
diphenhydramine	H ₁ blocker
chlorphentermine	sympathomimetic
fluoxetine	selective serotonin reuptake inhibitor
ammonium chloride	alkalinizing agent
amitriptyline	tricyclic antidepressant
propranolol	beta-blocker
chlorpromazine	dopamine antagonist
perhexaline	carnitine palmitoyltransferase inhibitor
amantadine	indirect dopamine agonist
gentamicin	aminoglycoside antibiotic

apeutically induced by cytotoxic agents. Tumor-selective chemotherapeutic regimens (such as emodin/arsenic trioxide) that preferentially induce heightened oxidative stress in cancer cells might exhibit additional efficacy if coadministered with chloroquine.⁴⁵ Similarly, chloroquine may prove beneficial in the treatment of tumors characterized by phenotypic autooxidations, such as melanoma.^{16,33,35} Both pre- and posttreatment, malignant melanoma cells exhibit heightened oxidative stress.^{23,35,43} Preclinically, chloroquine increases cytotoxicity in melanoma cell lines when administered alone or following radiation or hyperthermia.¹⁴ Therefore, chloroquine therapy might prove beneficial in the treatment of malignant melanoma.

Because of its ability to induce oxidative stress in *Plasmodium*, chloroquine has been used in the treatment of malaria for nearly half a century. Other chemically and pharmacologically diverse agents, such as artemisinin and tempol, demonstrate plasmodicidal and tumoricidal activities, providing evidence for a link between therapeutic effect and the creation of oxidative stress. These prooxidant activities probably explain the beneficial effects noted with chloroquine in the treatment of GBM with traditional radiotherapy. By selectively promoting tumor oxidative stress, chloroquine could prove beneficial in the treatment of other tumor types such as malignant melanoma, or following chemotherapy. Other selective redox agents, such as tempol and artemisinin, should be investigated clinically for their possible therapeutic benefit when coadministered with traditional GBM regimens.

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