International Journal of Radiation Oncology biology • physics

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Biology Contribution

Chloroquine Improves Survival and Hematopoietic Recovery After Lethal Low-Dose-Rate Radiation

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Received Oct 19, 2011, and in revised form Dec 17, 2011. Accepted for publication Jan 6, 2012

Summary

Chloroquine can abrogate the lethal cellular effects of low-dose-rate radiation *in vitro* by activating the ataxia-telangiectasia mutated (ATM) protein. Chloroquine administration before radiation has a significant effect on early engraftment of bone marrow cells and on the survival of normal but not ATM null mice, strongly suggesting that the *in vivo* effect, like the *in vitro* effect, is ATM dependent.

Purpose: We have previously shown that the antimalarial agent chloroquine can abrogate the lethal cellular effects of low-dose-rate (LDR) radiation *in vitro,* most likely by activating the ataxia-telangiectasia mutated (ATM) protein. Here, we demonstrate that chloroquine treatment also protects against lethal doses of LDR radiation *in vivo*.

Methods and Materials: C57BL/6 mice were irradiated with a total of 12.8 Gy delivered at 9.4 cGy/hour. ATM null mice from the same background were used to determine the influence of ATM. Chloroquine was administered by two intraperitoneal injections of 59.4 μg per 17 g of body weight, 24 hours and 4 hours before irradiation. Bone marrow cells isolated from tibia, fibula, and vertebral bones were transplanted into lethally irradiated CD45 congenic recipient mice by retro-orbital injection. Chimerism was assessed by flow cytometry. *In vitro* methylcellulose colony-forming assay of whole bone marrow cells and fluorescence activated cell sorting analysis of lineage depleted cells were used to assess the effect of chloroquine on progenitor cells.

Results: Mice pretreated with chloroquine before radiation exhibited a significantly higher survival rate than did mice treated with radiation alone (80% vs. 31%, $p = 0.0026$). Chloroquine administration before radiation did not affect the survival of ATM null mice ($p = 0.86$). Chloroquine also had a significant effect on the early engraftment of bone marrow cells from the irradiated donor mice 6 weeks after transplantation (4.2% vs. 0.4%, $p = 0.015$).

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Conflict of interest: none.

Supported by the American Society of Hematology Scholar Award, American Society for Clinical Oncology Young Investigator Award, American Association for Cancer Research-Astellas USA Fellowship for Basic Cancer Research to A.A.M.; The National Institutes of Health (R01CA127574, P01CA015396, K23CA107040), the Gabrielle's Angel Foundation for Cancer Research, the Sidney Kimmel Foundation for Cancer Research, and the Goodwin Foundation to (W.M.); The National Institutes of Health (R01CA71387 and P30CA21765), and the American-Lebanese-Syrian Associated Charities of St. Jude Children's Research Hospital to M.B.K.; and NIH/NCI P50 CA58236 Prostate Cancer SPORE to T.L.D.

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Supplementary material for this article can be found at www.redjournal.org.

Int J Radiation Oncol Biol Phys, Vol. 84, No. 3, pp. 800–806, 2012
0360-3016/$ - see front matter
doi:10.1016/j.ijrobp.2012.01.026

Chloroquine may serve as a useful drug for protection against the harmful effects of low-dose-rate radiation.

Conclusion: Chloroquine administration before radiation had a significant effect on the survival of normal but not ATM null mice, strongly suggesting that the *in vivo* effect, like the *in vitro* effect, is also ATM dependent. Chloroquine improved the early engraftment of bone marrow cells from LDR-irradiated mice, presumably by protecting the progenitor cells from radiation injury. Chloroquine thus could serve as a very useful drug for protection against the harmful effects of LDR radiation.

Keywords: Low-dose-rate radiation, Chloroquine, Hematopoietic progenitor cells, Ataxia telangiectasia mutated, Ataxia-telangiectasia mutated protein activation

Introduction

The biologic effects of ionizing radiation have been studied for more than a century, but it was not until deployment of the atomic bomb in 1945 that the clinical manifestations of total body irradiation (TBI) were fully realized. After acute radiation exposure, hematopoietic defects in particular are observed and are an important cause of death (1, 2). Lethal radiation exposure, either through accidents or possible acts of terrorism, remains a threat with the potential to affect large populations. Rather than acute radiation exposure, these exposures are likely to occur over protracted time periods, and the rate of radiation exposure is an important factor in resulting cellular toxicity. Biologic responses to lower dose rates vary depending on the cell type and the dose rate. Previous studies have found that the delivery of radiation at a low dose rate (LDR) may result in greater or lesser amounts of cell killing *in vitro* compared with equivalent doses delivered at higher dose rates (3, 4). These differences in cell survival are thought to result from alterations in the cell cycle and/or repair in radiation-induced injury (5, 6). Ataxia-telangiectasia mutated protein (ATM) is one of the key proteins involved in the response of mammalian cells to radiation-induced injury and is activated by autophosphorylation after DNA damage (7). Once activated, ATM subsequently phosphorylates other key proteins involved in the repair of DNA damage (8). The antimalarial agent chloroquine has been shown to activate ATM without inducing DNA injury, presumably by altering chromatin structure (7, 9). Intriguingly, ATM is not fully activated by LDR radiation, but the addition of chloroquine to cancer cells growing *in vitro* before LDR radiation exposure activates ATM and subsequently reduces cell death from LDR (10). Here, we examined whether chloroquine could similarly act as a radioprotective agent *in vivo,* and we treated mice with chloroquine before LDR radiation exposure. We found that chloroquine improved survival in normal but not ATM null mice. We also show that chloroquine enhanced recovery of hematopoietic progenitors responsible for early engraftment. These data expand our knowledge regarding the role of ATM in protection from radiation injury in mammals and highlight the possibility that drugs like chloroquine could be very useful as modulators of LDR radiation-induced injury.

Methods and Materials

Cell proliferation assay

Human fibroblast cells obtained from an ATM -/- patient were immortalized by use of hTERT (GM05823-hTERT ATM-/-). Immortalized wild-type human fibroblast cells (HFF-hTERT ATM+/+) were used as control. Cells were cultured in Dulbecco's Modified Eagle Media (DMEM) with 10% fetal bovine serum and treated with 48 μg/mL chloroquine for 4 hours, then washed with phosphate-buffered saline (PBS) and cultured in 10 mL fresh medium. Flasks were gassed with 5% CO_2, sealed, and irradiated in an incubated LDR irradiator with a ^{137}Cs source, which can be attenuated to produce various LDRs (3) for 42.5 hours at 37°C for a total radiation exposure of 4 Gy at a rate of 9.4 cGy/hour. Cell proliferation was assessed by use of Cell titer Blue (Promega, Madison, WI).

Mice

Male C57BL/6-CD45.2 mice (Harlan Laboratories, Indianapolis, IN) were used as bone marrow donors, and female C57BL/6-CD45.1 mice (National Cancer Institute) were used as transplant recipients. Male C57BL/6 ATM null mice (St. Jude Children's Research Hospital, Memphis, TN) were used in experiments to determine the influence of ATM in death induced by LDR TBI. ATM status was confirmed by polymerase chain reaction of mouse genomic DNA. All the mice were used at 4 to 6 weeks of age and were housed under specific pathogen-free conditions in an accredited facility at the Johns Hopkins University. All experiments were conducted according to protocols approved by the Johns Hopkins University Institutional Animal Care and Use Committee.

Radiation exposure and chloroquine administration

C57BL/6 donor mice were exposed in an LDR irradiator with a ^{137}Cs source attenuated to produce a dose rate of 9.4 cGy/hour in a custom-built insulated chamber approved for small animal exposure. Wild-type mice were treated for 136 hours for a TBI of 12.8 Gy. ATM null mice were treated for 96 hours for a TBI of 9 Gy, given their greater radiosensitivity (11). Bone marrow recipient mice were conditioned with 10 Gy given as two 5-Gy fractions at 30 Gy/hour, 4 hours apart, before bone marrow transplantation. Chloroquine (Sigma-Aldrich, St. Louis, MO) was dissolved in PBS, filter sterilized, and administered by two intraperitoneal injections of 3.5 mg/kg of body weight, 24 and 4 hours before LDR radiation exposure. The dose of chloroquine administered to the mice was previously determined in dose—response experiments (12).

Bone marrow transplantation

Donor mice were killed by cervical dislocation immediately after LDR radiation exposure. Bone marrow cell suspensions were prepared by crushing bones of the tibia, fibula, and vertebrae with a mortar and pestle in sterile PBS and then passed through a 70-μm filter. Bone marrow cellularity was determined by use of a Coulter counter. Whole bone marrow cell suspensions in PBS