**In vitro and In vivo Radiosensitization Induced by the Ribonucleotide Reductase Inhibitor Triapine (3-Aminopyridine-2-Carboxaldehyde-Thiosemicarbazone)**

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**Abstract**

**Purpose:** Because ribonucleotide reductase (RR) plays a role in DNA repair, it may serve as a molecular target for radiosensitization. Unlike previously investigated RR inhibitors, Triapine potently inhibits both RR holoenzymes. Therefore, the effects of Triapine on tumor cell radiosensitivity were investigated.

**Experimental Design:** The effects of Triapine on the *in vitro* radiosensitivity of three human tumor cell lines and one normal cell line were evaluated using a clonogenic assay. Growth delay was used to evaluate the effects of Triapine on *in vivo* tumor radiosensitivity. The levels of the RR subunits were determined using immunoblot analysis and DNA damage and repair were evaluated using γH2AX foci.

**Results:** Exposure of the tumor cell lines to Triapine before or immediately after irradiation resulted in an increase in radiosensitivity. In contrast, Triapine enhanced the radiosensitivity of the normal fibroblast cell line only when the exposure was before irradiation. There were no consistent differences between cell lines with respect to the expression of the RR subunits. Whereas Triapine had no effect on radiation-induced γH2AX foci at 1 hour, the number of γH2AX foci per cell was significantly greater in the Triapine-treated cells at 24 hours after irradiation, suggesting the presence of unrepaired DNA damage. Triapine administration to mice bearing tumor xenografts immediately after irradiation resulted in a greater than additive increase in radiation-induced tumor growth delay.

**Conclusions:** These results indicate that Triapine can enhance tumor cell radiosensitivity *in vitro* and *in vivo* and suggest that this effect involves an inhibition of DNA repair.

Radiotherapy continues to be a primary cancer treatment modality. Recent strategies aimed at increasing its efficacy have focused on targeting the molecules and processes that serve as determinants of cellular radiosensitivity. Among the fundamental processes that influence radiation-induced cell death is DNA repair; a critical molecule in this process is ribonucleotide reductase (RR). RR catalyzes the reduction of ribonucleotides to deoxyribonucleotides and thus provides an essential component for DNA synthesis and repair. RR is composed of two homo-dimer subunits (1). The R1 subunit (composed of two molecules of hRRM1) contains the ribonucleotide binding sites and allosteric effector sites. The R2 subunit, which contains a non-heme iron complexed with a tyrosyl free radical and is essential for catalytic activity, was initially defined as a homodimer of hRRM2. However, Tanaka et al. (2) recently identified the hRRM2 homologue, p53R2, which can serve as a functional alternative to hRRM2 as the R2 subunit (3). The circumstances that dictate whether the R2 subunit of RR is composed of hRRM2 or p53R2 have not been completely defined.

With respect to radiosensitivity, Kuo et al. (4) showed that whereas the overexpression of the R1 subunit has no effect on radiosensitivity, overexpression of the R2 subunit (in the form of hRRM2) protects against radiation-induced cell death, consistent with the R2 subunit of RR serving as a potential target for radiosensitization. Although a number of agents have been developed as RR inhibitors, hydroxyurea has received the most attention in preclinical and clinical studies. Hydroxyurea quenches the tyrosyl radical thereby destabilizing the iron center of hRRM2 with a resulting loss of RR enzymatic activity (5). A number of groups have reported that hydroxyurea can enhance the radiosensitivity of tumor cell lines (6-9). However, the combination of this RR inhibitor with radiotherapy has achieved only limited success in clinical trials (10). Recently, Shao et al. (11) showed that although hydroxyurea...
inhibits the activity of RR when the R2 subunit consists of hRRM2, it was relatively ineffective at inhibiting RR activity when the R2 subunit was composed of p53R2. In contrast, Triapine (3-aminopropyridine 5-carboxaldehyde thiosemicarbazone) inhibited RR activity when the R2 subunit was p53R2 or hRRM2 (11). Moreover, Triapine is an iron chelator (12) and a considerably more potent RR inhibitor than hydroxyurea (13).

To gain insight into the potential of combining Triapine with radiotherapy in the treatment of solid tumors, we have evaluated the effects of this RR inhibitor on the radiosensitivity of three human tumor cell lines. In this study, Triapine was found to enhance the radiosensitivity of the three tumor cell lines when delivered 16 hours before or immediately after irradiation. This sensitization was associated with an inhibition of the repair of radiation-induced DNA double-strand breaks as measured by γH2AX foci. In contrast to tumor cells, the radiosensitivity of a normal human fibroblast cell line was only enhanced when Triapine was provided before and not after irradiation. Finally, Triapine-induced tumor cell radiosensitization was extended to in vivo models using s.c. xenografts. The data presented thus suggest that the RR inhibitor Triapine may be an effective radiosensitizer suitable for clinical evaluation.

Materials and Methods

Cell lines and treatment. Three human tumor cell lines were evaluated: U251 (glioma), PSN1 (pancreatic carcinoma), and DU145 (prostate carcinoma). In addition, the nonimmortalized normal human fibroblast cell line MRC5 was evaluated. All cell lines except for U251 were obtained from American Type Culture Collection (ATCC, Manassas, VA); U251 was obtained from the DCTD Tumor Repository (National Cancer Institute, Frederick, MD). The tumor cell lines were grown in RPMI 1640 (Life Technologies, Rockville, MD) containing glutamate (5 mmol/L) and 5% fetal bovine serum and maintained at 37°C in an atmosphere of 5% CO2 and 95% room air. MRC-5 cells were grown in Earle’s MEM containing 10% fetal bovine serum as described by ATCC. The plating efficiency of each cell line was ≥50%. Triapine, provided by Vion Pharmaceuticals, Inc. (New Haven, CT), was dissolved in DMEM to a stock concentration of 1 mmol/L and stored at −80°C. For in vitro administration, Triapine was suspended as a fine particulate in saline. Cultures were irradiated using a Pantak (Solon, OH) X-ray source at a dose rate of 1.55 Gy/min.

Clonogenic survival assay. Cultures were trypsinized to generate a single-cell suspension and a specified number of cells were seeded into each well of six-well tissue culture plates. After allowing cells time to attach (6 hours), in the preirradiation exposure protocol, Triapine or the vehicle control was added at specified concentrations and the plates were irradiated 16 hours later. Immediately after irradiation, the growth medium was aspirated and fresh medium was added. To determine the effects of Triapine administered after irradiation, 6 hours after seeding into the six-well plates, cells were irradiated and Triapine was added immediately afterwards. The postirradiation Triapine exposure period was 16 hours; after which, the medium was aspirated and fresh growth media was added. Ten to twelve days after seeding, colonies were stained with crystal violet, the number of colonies containing at least 50 cells was determined, and the surviving fractions were calculated. Survival curves were generated after normalizing for the cytotoxicity induced by Triapine alone. Data presented are the mean ± SE from at least three independent experiments.

Cell cycle phase analysis. Evaluation of cell cycle phase distribution was done using flow cytometry. Cells were initially seeded into 10-cm dishes and exposed 16 hours later to the specified Triapine concentration for 16 hours. All cultures were subconfluent at the time of collection. Cultures were collected for fixation, stained with propidium iodide, and analyzed using flow cytometry as previously described by the Clinical Services Program at National Cancer Institute-Frederick (14).

Immunoblot analysis. Proteins comprising RR were evaluated using immunoblot analysis. Actively dividing cultures were rinsed with ice-cold PBS and scraped into lysis buffer (50 mmol/L Tris-HCl, 150 mmol/L NaCl, 1 mmol/L EDTA, 1% NP40), vortexed, centrifuged at 4°C, and the supernatant protein was quantified using a Bio-Rad (Hercules, CA) protein assay. Protein extracts (20 μg) were separated on 10% SDS-PAGE and transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA) in Tris-glycine buffer with 20% methanol. Membranes were blocked with 5% milk in TBS-Tween 20 solution, rinsed, and antibodies to hRRM1, hRRM2, or p53R2 (Santa Cruz Biotechnology, Santa Cruz, CA) and to actin (Chemicon, Temecula, CA) were applied. Chemiluminescent detection of the primary antibodies was done using the enhanced chemiluminescence plus Western Blotting Detection System (Amersham Biosciences, Little Chalfont, United Kingdom) and a Fuji LAS-3000 (Stamford, CT) was used for visualization and image acquisition.

Immunofluorescent staining for γH2AX. Cells were grown and treated in chamber slides. At specified times, medium was aspirated and cells were fixed in 4% paraformaldehyde/PBS for 10 minutes at room temperature. After aspiration, cells were exposed to 0.1% NP40 in PBS for 15 minutes on ice followed by 5% goat serum/1% bovine serum albumin in PBS for 1 hour at room temperature. Cells were then washed in PBS twice and the anti-γH2AX monoclonal antibody (Upstate Biotechnology, Lake Placid, NY) was added at a dilution of 1:100 in 1% bovine serum albumin for 1 hour. Cells were again washed twice in PBS before incubating in the dark with a FITC-labeled secondary antibody at a dilution of 1:100 in 1% bovine serum albumin for 1 hour. The secondary antibody solution was then aspirated and the cells were washed twice in PBS. Cells were then incubated in the dark with 4′,6-diamidino-2-phenylindole (1 μg/ml) in PBS for 30 minutes, washed twice, and coverslips mounted with an anti-fade solution (Dako Corp., Carpinteria, CA). Slides were examined on a Leica DMRXA fluorescent microscope (Wetzlar, Germany). Images were captured by a Photometrics Sensys CCD camera (Roper Scientific, Tuscon, AZ) and imported into IP Labs image analysis software package (Scanalytics, Inc., Fairfax, VA). For each treatment condition, γH2AX foci were determined in at least 50 cells.

Tumor growth delay. Six-week-old, female athymic nude mice (NCr nu/nu; National Cancer Institute Animal Production, Frederick, MD) were housed in filter-topped cages and provided autoclaved feed and hyperchlorinated water supplemented with amoxicillin (0.34 mg/ml) ad libitum. To generate tumor xenografts, 5 × 105 PSN1 or U251 tumor cells were implanted s.c. on the lateral aspect of the right leg. Tumors were irradiated locally using a Pantak irradiator with animals restrained in a custom lead jig. When tumors reached 172 mm3 (7 × 7 mm), Triapine (suspended in saline) or vehicle control was administered by i.p. injection; 6 hours later, tumors were irradiated. Alternatively, tumors were irradiated first and Triapine administered immediately after radiation. To obtain growth curves, perpendicular diameter measurements of each tumor were collected every 2 to 3 days with calipers; tumor volumes were calculated using the formula (L × W2) / 2 (ref. 15). Tumors were measured until the mean group tumor volume was >2,000 mm3. Each experimental group contained six mice and the untreated group contained 10 mice; absolute growth delays are expressed as the mean volume ± SE of each group (16). All animal studies were conducted in accordance with the principles and procedures outlined in the U.S. Public Health Service Guide for the Care and Use of Laboratory Animals in an Association for Assessment and Accreditation of Laboratory Animal Care International—approved facility under an approved animal protocol.

Results

The hypothesized influence of Triapine on tumor cell radiosensitivity was tested using three human tumor cell lines
of different histologic origins: DU145 (prostate carcinoma), U251 (glioma), and PSN1 (pancreatic carcinoma). For in vitro evaluations, DU145 and U251 cells were exposed to 5 μmol/L and PSN1 to 3 μmol/L Triapine, concentrations that after 16 hours reduced survival by ~50% (see below). To evaluate the effects of Triapine on tumor cell radiosensitivity when given before irradiation, cultures were exposed to Triapine for 16 hours, irradiated, rinsed, and fed Triapine-free media; colonies were counted 10 to 12 days later and survival curves generated after normalizing for the cytotoxicity induced by Triapine alone. As illustrated in Fig. 1A to C (pre-IR), exposure to Triapine before irradiation resulted in a significant increase in the radiosensitivity of each of the tumor cell lines with dose enhancement factors at a surviving fraction of 0.10 of 1.8, 1.5, and 1.6 for DU145, U251, and PSN1, respectively. To determine whether this radiosensitization was schedule dependent, survival curves were generated for tumor cell lines treated with Triapine immediately after radiation exposure. Cultures were irradiated, Triapine added, and 16 hours later, cultures were fed with Triapine-free medium. Colonies were counted 10 to 12 days later and survival curves generated after normalizing for the cytotoxicity induced by Triapine alone. As shown in Fig. 1A to C (post-IR), delivery of Triapine after irradiation also resulted in an enhancement in radiosensitivity for each of the tumor cell lines with dose enhancement factors similar to those induced by preirradiation treatment. Thus, the data presented in Fig. 1 indicate that Triapine exposure before or after irradiation results in tumor cell radiosensitization. The 16-hour exposure to Triapine alone, which was used in both the pre- and postirradiation protocols, resulted in surviving fractions of 0.68 ± 0.15, 0.53 ± 0.08, and 0.33 ± 0.16 in DU145, U251, and PSN1 cells, respectively.

Because inhibition of RR would be expected to modify cell cycle phase distribution, which may play a role in the radiosensitization induced when Triapine was given before irradiation (pre-IR), flow cytometry was used to determine the effects of Triapine on the cell cycle phase distribution of each of the tumor cell lines (Fig. 2). Although Triapine reduced the percentage of DU145 cells in S phase, there was only a minor effect, if any, on the S-phase population in U251 and PSN1 cells. In each of the tumor cell lines, however, Triapine

![Fig. 1. The effects of Triapine on tumor cell radiosensitivity. Cells were exposed to the designated concentrations of Triapine for 16 hours either before (pre-IR) or beginning immediately after (post-IR) irradiation. Cells were fed fresh growth media immediately after irradiation in the pre-IR groups or after the 16-hour Triapine exposure in the post-IR groups. Colony-forming efficiency was determined 10 to 12 days later and survival curves were generated after normalizing for cell killing by Triapine alone. A, DU145 (5 μmol/L); B, U251 (5 μmol/L); C, PSN1 (3 μmol/L). Points, mean of three independent experiments; bars, SE.](image1)

![Fig. 2. Effects of Triapine on cell cycle phase distribution. U251, DU145, and PSN1 tumor cell lines were exposed to Triapine (5, 5, and 3 μmol/L, respectively) for 16 hours and then collected for analysis of cell cycle phase distribution using flow cytometry. Results from representative analyses.](image2)
increased the number of cells in G1 and slightly decreased the number of cells in G2-M. In that there were minimal consistent changes among the cell lines in cell cycle phase distribution after 16 hours of Triapine exposure, it seems that the redistribution of cells into a radiosensitive phase of the cell cycle does not account for the enhancement in radiation-induced cell killing. Moreover, redistribution would not account for the sensitization detected when Triapine was given after irradiation.

Inhibition of RR depletes deoxynucleotide triphosphate pools and inhibits DNA synthesis (6), which would presumably impair DNA repair. Given that the critical radiation-induced DNA lesion with respect to cell death is the double-strand break, the effects of Triapine exposure on radiation-induced γH2AX foci were evaluated. γH2AX expression has been established as a sensitive indicator of double-strand breaks induced by clinically relevant doses of ionizing radiation (17). Therefore, to determine the effects of Triapine on the induction and repair of double-strand breaks, γH2AX foci were evaluated in U251 cells, which served as a representative of the three tumor cell lines. U251 cells were exposed to Triapine for 16 hours, as in the cell survival experiments, irradiated with 2 Gy, fed with Triapine-free medium, and γH2AX foci were determined up to 24 hours. Exposure of cells to Triapine only for 16 hours resulted in a significant increase (P < 0.05) in the number of γH2AX foci, a level that was maintained for at least 24 hours after drug removal (Fig. 3). Irradiation (2 Gy) only induced a significant increase in the number of γH2AX foci as detected at 1 hour, which progressively declined up to 24 hours. At 1 hour after irradiation, the levels of γH2AX foci were similar between cells receiving radiation only and those that had received the Triapine/radiation combination, suggesting that Triapine had no effect on the initial level of radiation-induced double-strand breaks. Whereas the number of foci in the combination treatment was slightly elevated as compared with radiation only at 6 hours (not statistically significant), there was a clear increase in the number of foci remaining at 24 hours in cells that received the Triapine/radiation combination as compared with those receiving radiation only or those that had received Triapine only. These data suggest that Triapine inhibits the repair of radiation-induced double-strand breaks.

Ideally, a radiosensitizer would be selective for tumor over normal cells. To evaluate the effects of Triapine on the in vitro radiosensitivity of normal human cells, the nonimmortalized normal fibroblast line MRC5 was exposed to Triapine (5 μmol/L) for 16 hours before or after irradiation, following the same treatment protocol as applied to the tumor cell lines (Fig. 4). Exposure of MRC5 cells to Triapine alone resulted in a surviving fraction of 0.45 ± 0.03, similar to that of the tumor cells. As for the tumor cell lines, treatment of MRC5 cells with Triapine for 16 hours before irradiation (pre-IR) resulted in an increase in radiosensitivity with a dose enhancement factor of 1.7. However, in contrast to the tumor cells, exposure to Triapine after irradiation (post-IR) had no effect on the radiosensitivity of these normal cells. To determine whether the disparity between the normal and the tumor cell lines with respect to radiosensitization, when Triapine was delivered post-IR, could be attributed to different levels of the RR subunits, particularly hRRM2 and p53R2, immunoblot analyses were done. As shown by the immunoblots in Fig. 5, after accounting for differences in loading, there were no consistent differences in the levels of hRRM1, hRRM2, and p53R2 between the tumor cell lines and the normal fibroblasts.

To determine whether the radiosensitizing effects of Triapine could be extended to an in vivo model, we used U251 and PSN1 cells grown as xenografts in nude mice. Two protocols were followed: Triapine delivered 6 hours before radiation, which was based on the kinetics of DNA synthesis inhibition...
previously reported in an in vivo tumor model (18), and Triapine delivered immediately after irradiation, which was based on the in vitro data shown in Fig. 1. Specifically, mice bearing s.c. xenografts (172 mm³) were randomized into five groups: vehicle (saline); Triapine only (60 mg/kg); 4 Gy only; Triapine (60 mg/kg) administered 6 hours before 4 Gy; and Triapine (60 mg/kg) administered immediately after 4 Gy. Treatment was on the day of randomization. The growth rates of U251 and PSN1 tumors exposed to each treatment are shown in Fig. 6A and B, respectively. For each group, the time to grow from 172 mm³ (volume at the time of treatment) to 2,000 mm³ was calculated using the tumor volumes from the individual mice in each group (mean ± SE); these data were then used to determine the absolute growth delays (the time in days for tumors in treated mice to grow from 172 to 2,000 mm³ minus the time in days for tumors to reach the same size in vehicle-treated mice).

For U251 tumors (Fig. 6A), the absolute growth delays for the Triapine alone and radiation alone groups were 2.1 ± 1.50 and 5.0 ± 0.71 days, respectively. In mice receiving Triapine followed 6 hours later by 4 Gy (pre-IR), the growth delay was 8.3 ± 0.98 days, which is only slightly greater than an additive response. However, the growth delay in mice administered Triapine immediately after 4 Gy (post-IR) was 15.3 ± 1.17 days, which is greater than the sum of the growth delays caused by Triapine alone and radiation alone. To obtain a dose enhancement factor comparing the tumor radioreponse in mice with and without Triapine treatment, the normalized tumor growth delays were determined, which accounts for the contribution of Triapine to tumor growth delay induced by the combination treatment. Normalized tumor growth delay was defined as the time in days for tumors to grow from 172 to 2,000 mm³ in mice exposed to the combined modality minus the time in days for tumors to grow from 172 to 2,000 mm³ in mice treated with Triapine only. The dose enhancement factors, obtained by dividing the normalized tumor growth delay in mice treated with the radiation/Triapine combination by the absolute growth delay in mice treated with radiation only, were 1.2 and 2.6 for Triapine delivered pre- and postirradiation, respectively. Thus, whereas Triapine delivered before radiation has little effect, the RR inhibitor administered after irradiation clearly enhances radiation-induced tumor growth delay.

Similar results were obtained for PSN1 xenografts (Fig. 6B), except that Triapine was delivered before radiation seemed to be more effective in this tumor model. Specifically, the absolute growth delays for Triapine alone and radiation alone were 0.9 ± 0.90 and 3.8 ± 0.63 days, respectively. In mice receiving Triapine followed 6 hours later by 4 Gy (pre-IR), the growth delay was 9.7 ± 1.11, and in mice administered Triapine immediately after 4 Gy (post-IR), the growth delay was 17.4 ± 1.40 days; each is greater than the sum of the growth delays caused by Triapine alone. However, as for U251 tumors, Triapine delivered after irradiation (dose enhancement factor, 4.3) was more effective than when Triapine was administered before irradiation (dose enhancement factor, 2.3). Thus, whereas these data extend the in vitro results shown in Fig. 1 to two in vivo tumor models, they also indicate that Triapine administered after irradiation is a more effective protocol for enhancing radiation-induced tumor growth delay.

Fig. 6. The effects of Triapine on radiation-induced tumor growth delay. When tumors reached 172 mm³ in size, mice were randomized into five groups: vehicle, Triapine (60 mg/kg delivered via i.p. injection), radiation (4 Gy), Triapine administered 6 hours before 4 Gy (pre-IR), and Triapine administered immediately after 4 Gy (post-IR). A, U251; B, PSN1. Points, mean tumor volumes of each group; bars, SE.
Discussion

The potential of RR serving as a target for tumor cell radiosensitization was initially suggested by studies showing that hydroxyurea enhanced the radiosensitivity of a number of experimental tumor models (6–9). Based on such laboratory studies, a number of clinical trials have been done combining radiotherapy with hydroxyurea. Whereas there was the suggestion of improved outcome, a recent systematic review of eight such studies by Symonds et al. (10) indicated that including hydroxyurea in radiotherapy protocols provided little to no benefit. However, more recent studies have shown that not all RR inhibitors are equal. Hydroxyurea has a relatively low affinity to RR (19), especially when it includes p53R2 (11). Moreover, tumor cells have been shown to readily develop resistance to hydroxyurea (20). In contrast, Triapine is a considerably more potent RR inhibitor and is effective against hydroxyurea-resistant murine and human tumor cells (18, 21). Moreover, Triapine is equally effective against the hRRM2 and p53R2 components of the R2 subunit of RR (11), and it is the R2 subunit that has been shown to protect against radiation-induced cell death (4). Triapine has undergone phase I clinical trials and has been shown to have a favorable pharmacokinetic and toxicity profile (22–26). Thus, biochemical data and initial clinical evaluations have suggested that Triapine may be an effective radiosensitizing agent.

For predicting the clinical performance of new chemotherapeutic agents, Voskoglou-Nomikos et al. (27) suggested that results obtained from a panel of human tumor cell lines evaluated in vitro or as xenograft tumors are predictive of efficacy in phase II clinical trials. It may be possible to extrapolate this analysis to the development of clinically effective radiation modifiers. Towards this end, the effects of Triapine on the in vitro radiosensitivity of three human tumor cell lines originating from different histologic origins were determined. As shown, this RR inhibitor enhanced the in vitro radiosensitivity of each tumor cell line. Moreover, the radiosensitizing actions of Triapine were extended to xenograft models of U251 and PSN1 tumor cells (DU145 cells were not evaluated in vivo). Consistent with the data of Voskoglou-Nomikos et al. (27), the ability to enhance the radiosensitivity of a genetically disparate panel of tumor cells suggests that Triapine may have clinical applicability in combination with radiotherapy.

The antitumor effects of the Triapine/radiation combination, however, would seem to be schedule dependent. Whereas the in vitro exposure of the tumor cells to Triapine resulted in radiosensitization whether the drug was administered 16 hours before or immediately after irradiation, in the in vivo models Triapine was clearly more effective when delivered after irradiation than when delivered before. This would seem to be the result of pharmacodynamic differences between monolayer cultures and solid tumors. Finch et al. (18) reported that for murine L1210 leukemia grown in vivo, the i.p. injection of Triapine depressed DNA synthesis for 9 hours, which rapidly returned to control levels by 11 hours. In our studies of solid tumors, Triapine was delivered 6 hours before tumor irradiation. For U251 xenografts, the effect of Triapine on radiation-induced growth delay was minimal, and for PSN1 tumors there was an enhanced response but not to the degree observed when the drug was delivered after irradiation. It should be noted that the temporary inhibition of DNA synthesis induced by Triapine alone had little effect on tumor growth rate. The greater degree of tumor radiosensitization induced when Triapine was delivered immediately after irradiation is consistent with the rapid inhibition of DNA synthesis (within 1 hour) after in vivo delivery (18). However, at 6 hours after drug delivery, it seems that these cells grown as solid tumors had recovered or had begun to recover from the effects of Triapine. Thus, these results suggest that the likelihood for success of clinical protocols combining Triapine with radiotherapy would be greater when the drug is delivered after a fraction of radiation.

The mechanism of radiosensitization induced by RR inhibitors has been assumed to involve cell cycle synchronization and/or inhibition of DNA repair. Hydroxyurea exposure results in an accumulation of cells in G1 and preferentially kills cells in S phase (28). An apparent radiosensitization may occur because cells in S phase are more radiosensitive than those in G1. Triapine exposure resulted in an increase in the percent of cells in G1 in each of the tumor cell lines; however, a reduction in S phase was not consistently observed. Whereas these data do not conclusively eliminate a role for cell cycle specificity when Triapine is delivered 16 hours before irradiation, the radiosensitization induced when this RR inhibitor was administered after irradiation both in vitro and in vivo suggests a role for DNA repair. A Triapine-mediated inhibition of DNA repair is supported by the experiment quantifying γH2AX foci levels. At sites of radiation-induced double-strand breaks, the histone H2AX becomes rapidly phosphorylated (γH2AX), forming readily visible nuclear foci (17, 29). Although the specific role of γH2AX in DNA repair has not been clearly defined, recent reports indicate that the dispersal of γH2AX foci in irradiated cells correlates with the repair of double-strand breaks (30, 31). The results presented here, in which the expression of γH2AX in cells treated with the Triapine/radiation combination was similar to radiation exposure only at 1 and 6 hours but significantly greater at 24 hours, are thus suggestive of an inhibition of DNA repair. Moreover, these results are consistent with a role for RR in the repair of radiation-induced DNA damage.

In this study, the nonimmortalized fibroblast cell line MRC5 was used as a model of normal human cells. Whether the radioresponse of fibroblasts grown in monolayer culture reflects that of normal tissue is certainly questionable. However, fibroblasts do not undergo apoptosis after irradiation as do cells of hematopoietic or lymphatic origin and would seem to provide an in vitro model at least representative of the signaling and repair processes operative in the normal cells involved in late radiation-induced tissue injury. Exposure of the normal MRC5 cells to Triapine before irradiation resulted in an enhancement in radiation-induced cell killing, similar to that detected in the tumor cell lines. In contrast to the tumor cells, exposure of the normal fibroblasts to Triapine after irradiation had no effect on their radiosensitivity. The mechanism responsible for this differential effect of postirradiation exposure to Triapine on the radiosensitivity of tumor and normal cells remains to be determined. However, the potential for the selective sensitization of tumor cells when Triapine is delivered after irradiation should be of relevance to the design of clinical combination protocols.
References

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