High-Throughput Screening Identifies Two Classes of Antibiotics as Radioprotectors: Tetracyclines and Fluoroquinolones

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Abstract

Purpose: Discovery of agents that protect or mitigate normal tissue from radiation injury during radiotherapy, accidents, or terrorist attacks is of importance. Specifically, bone marrow insufficiency, with possible infection due to immunosuppression, can occur after total body irradiation (TBI) or regional irradiation and is a major component of the acute radiation syndrome. The purpose of this study was to identify novel radioprotectors and mitigators of the hematopoietic system.

Experimental Design: High-throughput screening of small-molecule libraries was done using viability of a murine lymphocyte line as a readout with further validation in human lymphoblastoid cells. The selected compounds were then tested for their ability to counter TBI lethality in mice.

Results: All of two major classes of antibiotics, tetracyclines and fluoroquinolones, which share a common planar ring moiety, were radioprotective. Furthermore, tetracycline protected murine hematopoietic stem/progenitor cell populations from radiation damage and allowed 87.5% of mice to survive when given before and 35% when given 24 h after lethal TBI. Interestingly, tetracycline did not alter the radiosensitivity of Lewis lung cancer cells. Tetracycline and ciprofloxacine also protected human lymphoblastoid cells, reducing radiation-induced DNA double-strand breaks by 33% and 21%, respectively. The effects of these agents on radiation lethality are not due to the classic mechanism of free radical scavenging but potentially through activation of the Tip60 histone acetyltransferase and altered chromatin structure.

Conclusions: Tetracyclines and fluoroquinolones can be robust radioprotectors and mitigators of the hematopoietic system with potential utility in anticancer radiotherapy and radiation emergencies. (Clin Cancer Res 2009;15(23):7238–45)

Total body irradiation (TBI) with 5 to 10 Gy doses results in an acute radiation syndrome with possible lethality due primarily to hematopoietic failure and/or infection caused by immune impairment (1). Indeed, immunohematopoietic cells are very sensitive to radiation, dying mainly in interphase by apoptosis (2).

The peaceful and military use of atomic power after World War II spurred efforts to find agents for the prophylaxis, mitigation, or treatment of radiation injury, efforts that have been reintensified recently by an increased threat of terrorist use of radiation sources. Numerous compounds have radioprotective effects (3, 4). Examples are tempol, antioxidant vitamins, and melatonin, with the best studied being the thiol Amifostine (WR2721). Most are free radical scavengers that reduce initial radiation-induced DNA damage and work best if added just before or at the time of irradiation. Because of this, and their poor toxicity profile, amifostine and similar compounds are not practical countermeasures in a radiation incident (5). More recently, targeting superoxide dismutase (3, 4) and activation of Toll-like receptor 5/NF-κB pathway by flagellin suggest that alternative approaches may be of value (6). Furthermore, certain cytokines such as granulocyte colony-stimulating factor, stem

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Translational Relevance

The sensitivity of the hematopoietic system to ionizing radiation results in adverse side effects following exposure and there is a lack of agents able to prevent or mitigate this damage. Our study using high-throughput screening of small-molecule bioactive compounds identified two major classes of antibiotics, specifically tetracyclines and fluoroquinolones, as being radioprotective of normal hematopoietic tissue both *in vitro* and *in vivo*. These drugs therefore have potential to improve the outcome of radiation exposure in several different scenarios, and as they do not seem to affect tumor responses, this may include cancer radiotherapy. Our findings suggest that the choice of antibiotics in settings of radiation exposure may benefit from consideration of more than purely antimicrobial criteria. In addition, the common structural moiety shown to be shared by both classes of antibiotics might serve as a lead scaffold for the discovery of better radioprotectors and mitigators.

cell factor, and granulocyte macrophage colony-stimulating factor can accelerate recovery of the hematopoietic system after TBI (3, 4). However, the dearth of agents with robust, prolonged efficacy, broad specificity, and minimal toxicity that could protect a large population in the event of a radiological emergency, or that could increase the radiotherapeutic benefit of cancer treatment, warrants further searches.

We chose an unbiased high-throughput screening approach to identify modulators of radiation response, with the hypothesis that effective agents might form classes that share molecular signatures (common chemical structures and biological pathways). Agents were given either before or after radiation to determine if they prevented or mitigated against radiation toxicity, respectively, or both. Radiation-induced apoptosis of a murine T lymphocyte cell line (Til1) was the primary screening endpoint and human lymphoblastoid cell lines (LCL) were used for validation of compounds that could act across species barriers. Finally, agents were tested for their ability to protect mice and their immunohematopoietic system following TBI.

From screening of 3,600 bioactive compounds with known biological activity, all members of two classes of antibiotics, tetracyclines and fluoroquinolones, 18 in number, stood out as possessing radioprotective properties. In general, these compounds had low toxicity and representative compounds could improve progenitor cell and whole-animal survival after lethal TBI. Some were effective *in vivo* even when given after TBI. We conclude that high-throughput screening, although unable to fully recapitulate many aspects of the complex *in vivo* acute radiation syndrome response, can be used to identify agents that modulate radiation responses.

Materials and Methods

Small-molecule libraries. Three thousand six hundred bioactive compounds from Prestwick, Biomol, and Spectrum (MicroSource Discovery Systems) libraries were tested at a 10 μmol/L final concentration in 1% DMSO using an automated Biomek FX Workstation (Beckman Coulter).

Cell lines and irradiation. A $CD4^+CD8^+$ murine T lymphocyte cell line (Til1; ref. 7) was cultured in DMEM with 10% fetal bovine serum, 2 mmol/L L-glutamine, 100 units/mL penicillin G, and 100 μg/mL streptomycin. Human LCLs derived from peripheral blood lymphocytes by transformation with EBV (8) were cultured as published (9). Cells were irradiated with a Mark I ^{137}Cs irradiator at a dose rate of 5 Gy/min.

High-throughput screening of libraries. Ten thousand Til1 cells were dispensed into each well of 384-well plates using a Multidrop384 (Thermo Scientific). To identify radioprotectors, cells were preincubated with compounds for 3 h before irradiation (2 Gy). For mitigators, cells were irradiated 1 h before compound loading. Cell viability was determined at 24 h post-irradiation by luminescence-based measurement of ATP production (ATPlite reagent; Perkin-Elmer) with a SpectraMax M5 microplate reader (Molecular Devices). Each compound was represented four times and the average was used for data processing. The Z' factor (10) for the assay was >0.5. To qualify for validation, the average values for the compound normalized to the vehicle controls had to be >130%.

Similarity and substructure analysis. Similarity and substructure searches were done on the Collaborative Drug Discovery platform. The entire library was ranked according to its structural similarity to a referenced hit based on the Tanimoto coefficient, excluding coefficients <0.7. Hits and nonhits within the library with similar structure were identified and a substructure analysis done to determine minimal elements.

Secondary screening with Annexin V/propidium iodide staining. Human LCLs were incubated for 2 h with the compounds at the final concentrations indicated and irradiated with 5 Gy, and 48 h later, apoptosis

Table 1. EC_{50}s for positive compounds

	EC_{50} (μmol/L)
Primary screening	
Prevention	
Norfloxacin	3.67
5′-AMP	6.05
Doxycycline	0.5
Tetracycline	0.33
Chlorotetracycline	1.15
Minocycline	0.24
Meclocycline	0.55
Ciprofloxacin	1.09
Moxifloxacin	0.8
Cyclopiazonic acid	2.67
Mitigation	
Linoleic acid	8.51
Scopolamine	9.86
Rifabutin	4.11
Vidarabine	7.83
Acivicin	1.13
Deoxyadenosine	8.23
Tilorone	2.49
Similarity analysis	
Prevention	
Rolitetracycline	4.03
Oxytetracycline	2.49
Methacycline	1.44
Gatifloxacin	2.19
Levofloxacin	2.19
Enoxacin	13.87
Flumequine	58.2
Lomefloxacin	27.77
Ofloxacin	12.55
Sarafloxacin	0.7

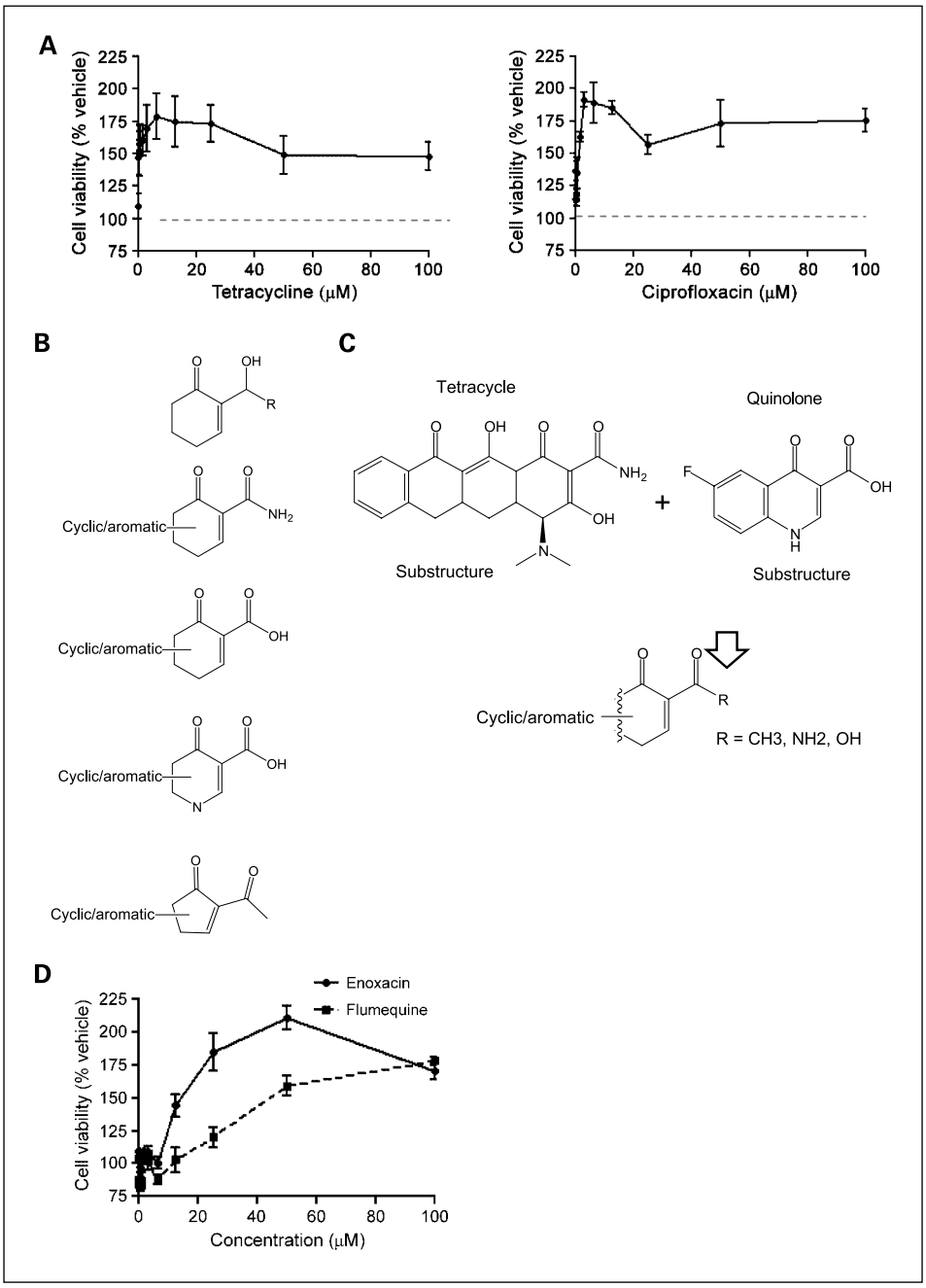

Fig. 1. Dose-response of representative radioprotectors and structure-activity relationship/substructure analysis. *A,* tetracycline and ciprofloxacin. *B,* the first substructure was present only in compounds that were negative in the initial screen (11 compounds in total). All parent compounds containing this substructure possessed no adjacent cyclic/aromatic planar character and were all conjugated at the acyl functionality (R) to various other molecules. The second structure was present in all 12 tetracyclines. No compound within the libraries screened contains the third substructure. Eighteen compounds contained the fourth substructure, categorized as the fluoroquinolone class, and one compound (cyclopiazonic acid) contained the fifth substructure. *C,* core substructures contained in all positive hits for tetracyclines and fluoroquinolones with a common structural theme shown. *D,* examples from similarity analysis (Table 1, column 2). The percent cell viability normalized to vehicle control value is plotted. Note that the decrease in protective activity is presumably due to drug toxicity.

was assessed using Annexin V/propidium iodide (BioVision) followed by flow cytometry.

Animal survival assay. C3Hf/Kam mice were bred and maintained in a strict defined-flora, pathogen-free environment in the American Association of Laboratory Animal Care–accredited animal facilities of the Department of Radiation Oncology, University of California at Los Angeles. The University of California at Los Angeles Animal Care and Use Committee approved all experiments, which were done in accordance with all local and national guidelines for the care and use of animals. Male mice, 10 to 15 weeks old, received 8 Gy TBI from a Gamma Cell 40 irradiator (^{137}Cs source; Atomic Energy of Canada) at a dose rate of 67 cGy/min. For radioprotection, drug or vehicle was injected intraperitoneally 24 and 1 h before TBI. For mitigation, they were given five times daily starting 24 h after TBI. Mice were monitored for 30 days using standard criteria for humane euthanasia as an endpoint.

Colony formation assay. Bone marrow cells were harvested from femurs of C3Hf/Kam mice (n = 4 per group) treated with vehicle, tetracycline, TBI with vehicle, or TBI with tetracycline. Water or tetracycline at 150 mg/kg was given intraperitoneally 24 and 1 h before TBI and bone marrow cells were collected 3 days later. RBC were lysed using ACK buffer (Lonza), bone marrow cells were resuspended in IMDM containing Methocult M3234 (StemCell Technologies) and 10 ng/mL recombinant murine granulocyte macrophage colony-stimulating factor (Invitrogen), and 2×10^4 bone marrow cells were plated into a 35 mm dish in triplicate. Colonies were counted after 9 days.

Clonogenic survival assay. Exponentially growing murine Lewis lung cancer cells were pretreated with tetracycline at 5, 10, or 20 μmol/L for 4 h, trypsinized, irradiated with 2, 4, and 6 Gy, and plated in 100-mm dishes in triplicate. After 10 days, colonies were stained

with crystal violet in 50% ethanol. Colonies consisting of >50 cells were counted to determine clonogenic survival.

γH2AX immunofluorescence. Human LCLs were collected after 18 h incubation with the indicated compound and irradiated with 2 Gy. Fixing and staining procedures were done as published (9).

Reactive oxygen species measurement. Intracellular reactive oxygen species were measured using 2′,7′-dichlorofluorescein diacetate (Invitrogen; refs. 11, 12). TiL1l cells were incubated with compound for 2 h, and 25 μmol/L 2′,7′-dichlorofluorescein diacetate probe was added for 1 h and irradiated with a high dose (10 Gy) to generate significant reactive oxygen species. Fluorescence was measured by flow cytometry (13).

Tip60 histone acetyltransferase assay. HeLa cells or LCLs were incubated with compounds for 4 h, extracts were prepared, and Tip60 was immunoprecipitated (Upstate Biotechnology). Precipitates were incubated with biotinylated histone H4 peptide and acetyl-CoA for 15 min (14), an aliquot was immobilized onto streptavidin plates, and acetylation was detected by ELISA using acetyl-lysine–specific antibody (Upstate Biotechnology).

Results

Primary screening and structure-activity relationships. Libraries of 3,600 bioactive compounds were screened at 10 μmol/L for their ability to cytoprotect Til1 cells. Pilot experiments determined that the most suitable experimental design was adding compounds either 3 h before or 1 h after 2 Gy, which reduced viability of vehicle-treated cells to ~30% at 24 h. Compounds were considered as possible candidates if they increased this value by 130%. When added before irradiation, 22 hits were obtained (0.61%); after irradiation, 18 hits (0.5%) were obtained. Hits were confirmed using the same screening assay over the dose range 195 nmol/L to 100 μmol/L. As a result, 10 (0.28%) and 7 (0.19%) compounds were chosen as the most reliable radioprotectors and mitigators, respectively ($P < 0.05$; Table 1, column 1). The EC_{50} of these compounds generally ranged between 0.2 and 10 μmol/L with a large therapeutic window of 1 to 2 logs.

Of the 10 compounds that radioprotected Til1 cells, 8 were tetracycline derivatives or fluoroquinolones (representatives in Fig. 1A). No other class of antibiotics were radioprotective, although the Spectrum library alone contains >194 bactericidals out of 2,000 compounds and there were large representations of β-lactam–based drugs such as penicillin G and ampicillin and macrolides such as erythromycin. To make sure that this was not simply a dosage effect, penicillin G, ampicillin, and erythromycin were additionally assayed at multiple doses up to 100 μmol/L and were negative ($P > 0.05$; data not shown). Radioprotection by antibiotics seems therefore to be a sole property of tetracyclines and fluoroquinolones and is obviously separate and distinct from their bactericidal properties.

All positive hits within and across libraries were computationally compared using the Tanimoto rule of similarity to identify possible structure-activity relationship and common substructures. The results are in Fig. 1B, with a general formula indicating a common cyclic/planar aromatic ring in Fig. 1C. Of interest is that reverse analysis of all active substructures within negative data yield the first substructure in Fig. 1B, in which all of the compounds do not possess the adjacent ring character and all are conjugated at the acyl functionality. The only other group of positive compounds with structural similarity is composed of three nucleotide derivatives, deoxyadenosine (mitigation), vidarabine (mitigation), and 5′-AMP (prevention), which has been reported previously to be radioprotective (refs. 15, 16; Table 1, column 1).

"Similarity searches" were also used to identify false-negative tetracyclines and fluoroquinolones in the libraries. Of the 10 tetracyclines, 5 had already been identified by the primary screening, and of the 12 fluoroquinolones, 3 had been recognized as hits. To determine if the others had been miscategorized, for example, because the dosage was suboptimal, or were true negatives, they were retested in the primary screen over a wider dose range. Only 10 of the 14 antibiotics were commercially available, but of these all 3 tetracyclines and all 7 fluoroquinolones retested as positive by the original criteria (cell viability >130%; $P < 0.05$, compared with the vehicle control; Table 1, column 2). Four of these had not been detected earlier because their EC_{50} was above the 10 μmol/L test dose (representatives in Fig. 1D). In other words, all 18 members of these two classes of antibiotics that could be tested were radioprotective.

Because each compound was tested for both preventative and mitigating activities, we were able to determine if there was any correlation between these activities and if any were active when given both before and after irradiation. There was a weak correlation of 0.344 for Prestwick, 0.177 for Spectrum, 0.524 for Biomol-enzyme, and 0.272 for Biomol-lipid libraries (Supplementary Fig. S1). The overall correlation was, however, statistically highly significant ($P < 0.001$) due to the large number of compounds. A few compounds fell outside the 95% confidence level ellipse in the upper quadrant, indicating that they had both preventative and mitigating activities. These will be the subject of another publication.

Secondary screening using human LCLs. For further validation, the ability of 20 radioprotectors from the primary screens (Table 1) to radioprotect EBV-transformed wild-type LCLs was assessed using Annexin V and propidium iodide staining. Ataxia telangiectasia LCLs were included because even moderately effective radioprotectors could be beneficial for these patients given their hypersensitivity to radiation (17). Overall, 10 of the 20 compounds showed activity (Table 2), which was remarkable because the cells were from a different species, were EBV-transformed B cells as opposed to nontransformed T cells, and were tested in a different assay using a different radiation protocol. However, there was no consistent pattern of ATM

Table 2. Secondary screening by Annexin V/propidium iodide staining % conferred reduction compared with vehicle controls

Compound (μmol/L)	Wild-type	Ataxia telangiectasia
Tetracycline (20)	19	NS
Norfloxacin (40)	28	28
Levofloxacin (40)	16	34
Doxycycline (10)	NS	31
Chlorotetracycline (20)	NS	48
Moxifloxacin (10)	NS	50
Ciprofloxacin (5)	21	NS
Flumequine (100)	25	37
Enoxacin (100)	18	NS
5′-AMP (20)	34	13

NOTE: Only the compounds with statistically significant reduction in apoptosis in wild-type or ataxia telangiectasia LCLs are shown ($P < 0.05$). NS, not significant.

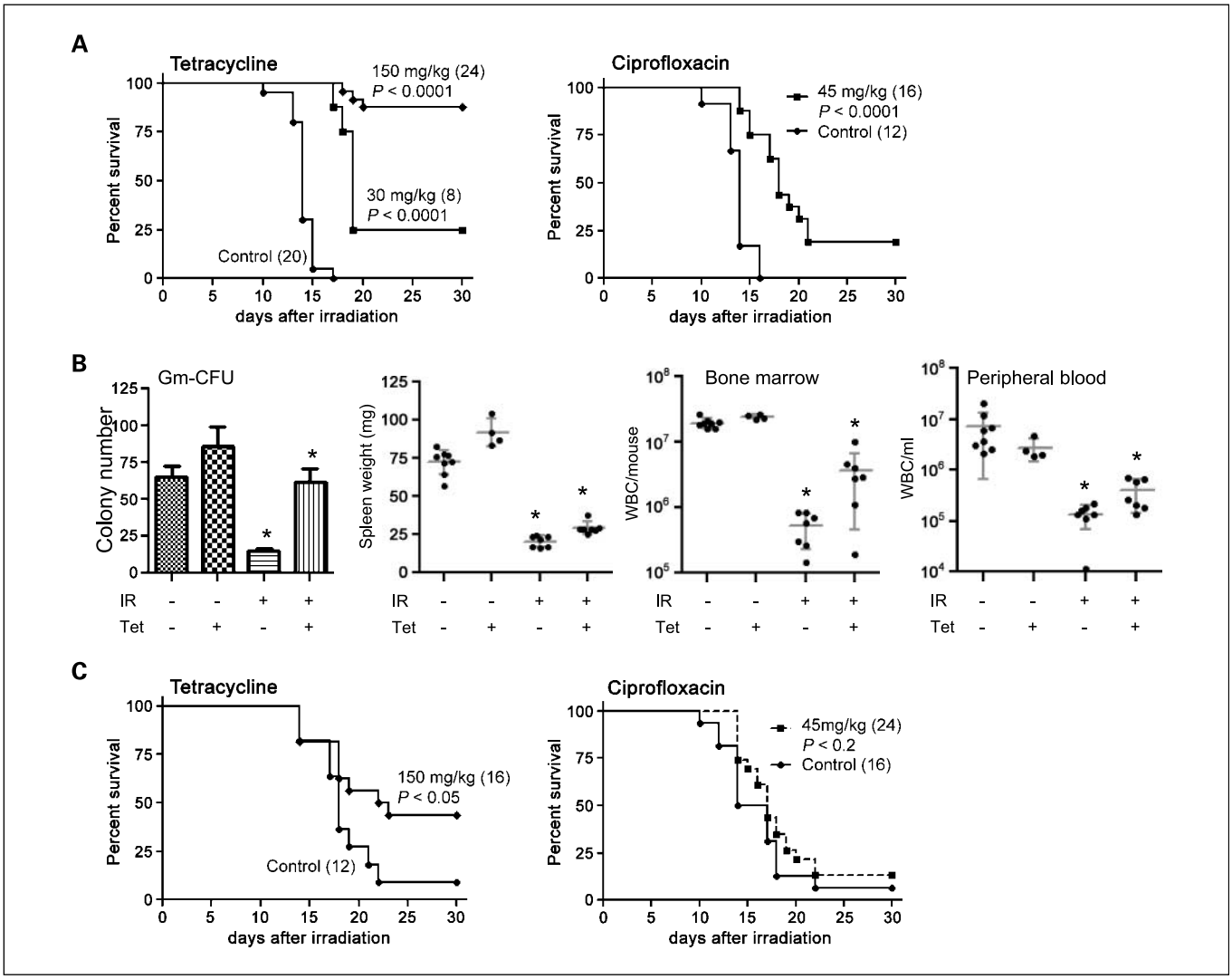

Fig. 2. Effect of tetracycline and ciprofloxacin *in vivo* against lethal TBI. *A,* two intraperitoneal injections of tetracycline or ciprofloxacin at 24 and 1 h before 8 Gy TBI protected mice from lethality. This effect was most prominent with tetracycline at 150 mg/kg (87.5% survival). *B,* the same schedule of tetracycline treatment at 150 mg/kg as in *A* protects the immunohematopoietic system from lethal TBI. Spleen weights and white cell counts were done 3 days later and bone marrow cells were pooled from 4 mice per treatment group for assessment of granulocyte/macrophage colony-forming units (*Gm-CFU*). Data from two separate experiments are shown (combined $n = 7$, except tetracycline-treated group: $n = 4$). *, $P < 0.05$ (IR versus IR + tetracycline). *C,* five daily injections of tetracycline starting 24 h after 8 Gy TBI improves animal survival ($P < 0.05$), whereas ciprofloxacin failed as a mitigator. Number of mice in each treatment group is in parentheses.

dependence of the potential radioprotective effect. Four of the 20 significantly reduced radiation-induced apoptosis ($P < 0.05$) in both wild-type and ataxia telangiectasia, 3 only in wild-type, and 3 only in ataxia telangiectasia. The data therefore speak to the universality of the effects of these compounds, but further studies are needed to explore the role of ATM in this form of radioprotection.

Tetracycline protects the immunohematopoietic system and allows mice to survive lethal TBI. Tetracycline and ciprofloxacin were chosen as representatives of the two classes of antibiotics for *in vivo* studies in part because they have been clinically most widely used and were active in both murine and human assays. When tetracycline (150 mg/kg) was given 24 and 1 h before a lethal dose of 8 Gy TBI, 87.5% of mice survived, whereas all vehicle-treated controls died ($P < 0.0001$; Fig. 2A). Tetracycline at 30 mg/kg and ciprofloxacin at 45 mg/kg showed some, but less, activity. The same tetracycline schedule that improved animal survival (150 mg/kg) caused an increase in hematopoietic stem/progenitor cells as shown by a granulocyte/macrophage colony formation assay, higher spleen weights, and higher WBC counts in bone marrow and peripheral blood after lethal TBI (Fig. 2B). Remarkably, when tetracycline was given as five daily injections (150 mg/kg) starting 24 h after 8 Gy TBI, survival was also significantly enhanced (Fig. 2C). Ciprofloxacin given at 45 mg/kg by the same schedule failed to mitigate radiation-induced lethality.

Tetracycline does not interfere with radiation treatment of mouse tumor cells. To determine if tetracycline would protect cancer as well as normal cells, it was added to murine Lewis lung cancer cells at doses equal or higher than those that radioprotected Til1 and human LCLs and a clonogenic survival was assessed after various radiation doses (Fig. 3). Tetracycline did not protect Lewis lung cancer cells from radiation treatment, indicating that this drug may have potential in cancer radiotherapy.

Tetracycline and ciprofloxacin protect LCLs from radiation-induced DNA double-strand breaks. Having confirmed the radioprotective activity of tetracycline and ciprofloxacin both *in vitro* and *in vivo*, we explored their mechanism of radioprotection. First, the incidence of radiation-induced DNA double-strand breaks (DSB) in wild-type LCLs after 2 Gy, as measured by phosphorylation of histone H2AX (γH2AX), was decreased by prior treatment with tetracycline (Fig. 4A and quantified in Supplementary Table S1) and with ciprofloxacin, chlorotetracycline, and moxifloxacin (Supplementary Table S1), suggesting that initial DNA DSB formation or repair was affected. Although there are no apparent redox centers within these structures, the inability of these compounds to act as free radical scavengers was confirmed by measuring levels of reactive oxygen species by flow cytometry in Til1 cells immediately after irradiation using 2′,7′-dichlorofluorescein (Fig. 4B).

The effect of tetracycline on the radiation-induced DNA damage downstream response was then assessed by Western blotting for phosphorylated forms of ATM, Chk2, DNA-PKcs, p53, and SMC1 proteins in the cell extracts of wild-type and ataxia telangiectasia LCLs following irradiation with 10 Gy (Supplementary Fig. S2). Tetracycline treatment did not robustly alter the phosphorylation status of these molecules; however, activation of a putative radiation target upstream of ATM protein, specifically Tip60 histone acetyltransferase (HAT), was affected. Tip60 HAT plays a central role in the DNA damage response being activated by ionizing radiation or bleomycin (14), the latter being used as the radiomimetic agent in our assay. All tested antibiotics upregulated Tip60 HAT activity strongly in HeLa cells (4- to 5-fold of control) to a level similar to that of bleomycin (Fig. 4C), and tetracycline and ciprofloxacin did the same in human wild-type LCLs but to a lesser degree (2- to 3-fold of control; Fig. 4D).

Discussion

Tetracyclines and fluoroquinolones are broad-spectrum antibiotics that act against gram-positive and gram-negative bacteria. Nonantimicrobial activities of tetracyclines include inhibiting inflammation, angiogenesis, and apoptosis as well as chelating divalent metal cations (18). Of possible relevance to this study, minocycline prevented neuronal cell apoptotic death in mice, reducing tissue injury and neurologic deficits (19). Minocycline was similarly shown to delay mortality in a transgenic mouse model of Huntington disease (20) and to hinder progression of amyotrophic lateral sclerosis in mice (21). Fluoroquinolones inhibit DNA gyrase (prokaryotic topoisomerase II) and topoisomerase IV in bacteria through direct chromosome binding, but certain members of this family also display activity against eukaryotic topoisomerase II. They can therefore be toxic to proliferating cells and are being explored as anticancer drugs (22). They have been documented as having anti-inflammatory properties, decreasing the synthesis of proinflammatory cytokines such as interleukin-1 and tumor necrosis factor (23, 24). Furthermore, fluoroquinolones including ciprofloxacin, sparfloxacin, and clinafloxacin have been reported to stimulate hematopoiesis and slightly prolong the survival of sublethally irradiated mice (25) while aiding survival of TBI mice transplanted with bone marrow cells by decreasing the systemic spread of bacteria (26). It should be noted that the mice used in our study have a limited flora and lack culturable gram-negative organisms. Because of this, and the direct radioprotection afforded murine T lymphocytes and human B lymphocytes *in vitro*, we believe that the antimicrobial action of these compounds does not contribute to our *in vivo* findings.

The common planar ring structure that is shared by tetracyclines and quinolones and absent from other classes of antibiotics might explain their common radioprotective activity and provide a lead scaffold for compounds with improved efficacy. How this structure holds the core radioprotective activity, however, needs further investigation. Free radical scavenging, which is a common attribute of most radioprotectors (3), is clearly not involved. Both tetracycline and ciprofloxacin did reduce radiation-induced γH2AX foci formation, although activation of downstream DNA damage signaling molecules such ATM, DNA-PKcs, Chk2, p53, and SMC1 did not seem to be robustly altered. We were able to show a radioprotective effect of some compounds in ataxia telangiectasia LCLs but not in others, and a clear picture did not emerge as to the ATM dependency. This may be because DNA DSBs in ataxia telangiectasia LCLs after irradiation are more extensive than in wild-type LCLs (data not shown) and more difficult to repair. However, these compounds did activate the HAT Tip60 (Fig. 4C and D), indicating that they might directly influence chromatin structure and DNA damage responses.

Tip60 is a key component in the remodeling of chromatin structure during the repair of DNA DSBs (27–30). Because the extent of chromatin condensation influences radiosensitivity (31, 32), and genetic or chemical inactivation of Tip60 increases the sensitivity of cells to DNA damage (14, 33), this could be a mechanism of radioprotection. Tip60 HAT recruitment and histone acetylation surrounding DSBs have been found to be mediated by the HAT cofactor Traap (34, 35). Traap depletion impairs DNA damage–induced H4 acetylation and recruitment of RAD51 and BRCA1, leaving γH2AX accumulation and ATM-dependent DNA damage signaling intact. This could explain why, although ATM is one target of Tip60 (14), ATM did not

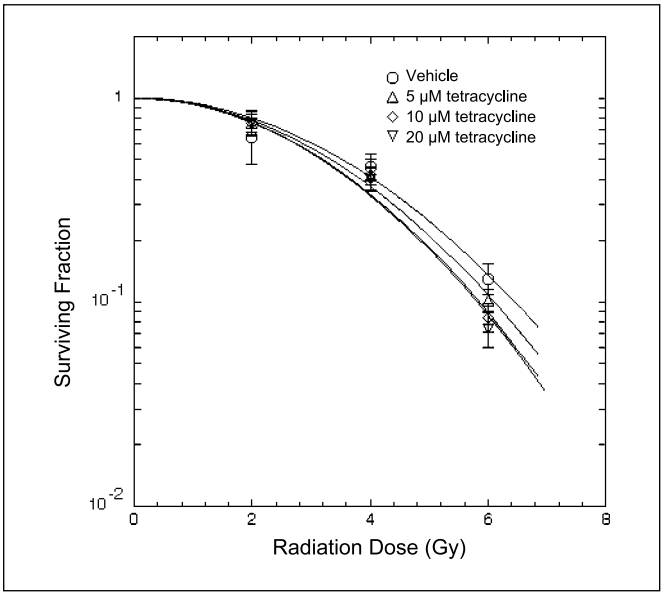

Fig. 3. Effect of tetracycline on the radiosensitivity of Lewis lung cancer cells. Clonogenic assays with different doses of tetracycline were done. The range of tetracycline doses used in this assay did not interfere with radiation treatment on Lewis lung cancer cells.

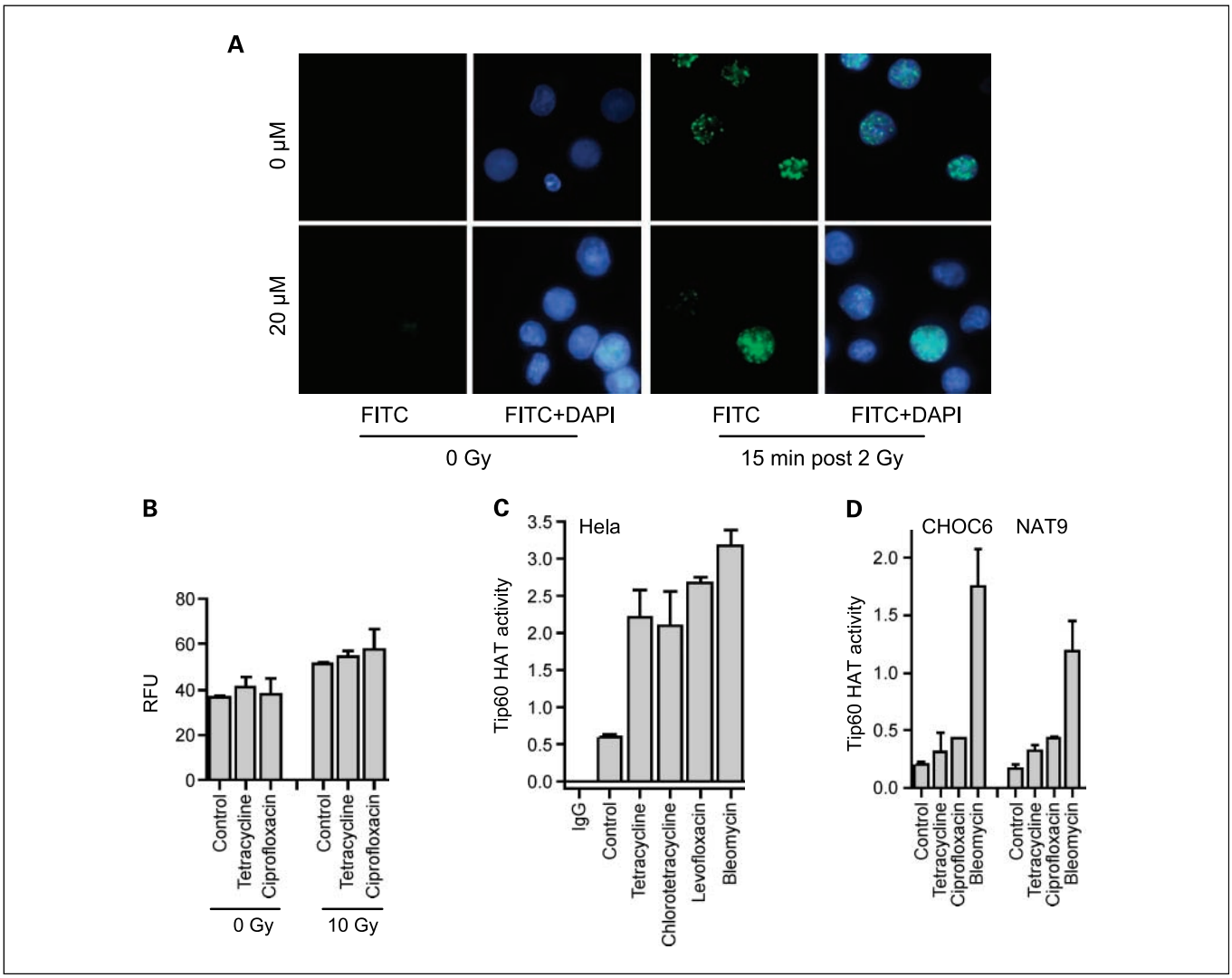

Fig. 4. Protection against radiation-induced DNA DSBs. *A,* effect of tetracycline on radiation-induced DNA DSBs was assessed by γH2AX immunofluorescence foci formation at 15 min after irradiation at 2 Gy in human wild-type LCLs. Tetracycline (20 μmol/L) reduced radiation-induced foci by 33%. *B,* tetracycline (10 μmol/L) or ciprofloxacin (5 μmol/L) did not reduce radiation-induced reactive oxygen species in Til1 cells. Tetracyclines and fluoroquinolone antibiotics stimulated Tip60 HAT activity in HeLa cells to the level similar to the radiomimetic agent bleomycin (*C*) and in two human wild-type LCLs (CHOC6 and NAT9) to a lesser degree (*D*). Bleomycin (5 μmol/L), tetracycline (25 μmol/L), ciprofloxacin (10 μmol/L), levofloxacin (10 μmol/L), and chlorotetracycline (10 μmol/L) were used for the HAT assay.

appear to be consistently activated in our study. On the other hand, the homologous DNA repair machinery involving RAD51 and BRCA1 seems to require Tip60-dependent chromatin relaxation (34, 35) that the intercalating properties of tetracycline and ciprofloxacin might induce. A similar mechanism was proposed for the action of chloroquine, which can act as a radioprotector, but this drug activates ATM (36, 37).

Our finding that tetracycline promotes survival of mice even if given after TBI raises questions as to whether it ameliorates persisting radiation damage (38), activates signaling pathways leading to accelerated recovery of the immunohematopoietic system, or mitigates by other mechanisms. Minocycline has recently been shown to inhibit release of the nonhistone DNA-binding high mobility group box-1 protein in oxygen-glucose–deprived PC12 cells and trigger p38 mitogen-activated protein kinase and extracellular signal-regulated kinases 1/2 prosurvival pathways (39), which leaves options open for further research.

Finally, it should be noted that antibiotics are already an important component of the treatment of radiation injuries (3, 40). Indeed, quinolone antibiotics and penicillin were attributed to reducing mortality in the Chernobyl nuclear accident (41). Our findings suggest that the choice of antibiotics in such emergencies, as well as in cancer patients receiving radiotherapy, could benefit from consideration of more than purely microbiological criteria because not all classes of antibiotics are active. Further, although tetracycline and ciprofloxacin have long been used in the clinic and there is no evidence of long-term deleterious effects, their ability to inhibit or enhance radiation carcinogenesis should be investigated because modulation of radiation responses could have either outcome depending on the mechanistic pathway through which they work.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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